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Biology and Ecology of Palmer Amaranth (Amaranthus palmeri)

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BIOLOGY AND ECOLOGY OF PALMER AMARANTH (*AMARANTHUS PALMERI*)

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ABSTRACT

Palmer amaranth (*Amaranthus palmeri* S. Wats.) is a troublesome weed of crops in southeastern United States. This research highlights studies on the biology and ecology of Palmer amaranth. Following dispersal in fall, Palmer amaranth seeds require high mean temperatures of 25 to 40 C for germination, which is not likely to occur in South Carolina until the following spring. With dormancy reduction over winter, seeds can germinate at high temperatures (≥25 C) and thermal amplitudes of 15 C during late spring (May) in the presence of light. A majority (>90%) of the non-dormant population in the soil seedbank emerge from early May through mid-July, with two to three peak emergence periods which often follow rainfall events. No difference in emergence was observed between no-tillage and shallow spring tillage situations.

Early canopy closure in drill-seeded soybean (18-cm row width) had a suppressive effect on the emergence of Palmer amaranth cohorts following early July. This is attributed to the decrease in photosynthetic active radiation (PAR) and red:far-red (R:FR) ratio experienced by seeds lying on the soil surface beneath the canopy in no-tillage systems. Seed germination during fall (August to November) is phytochrome-regulated, with germination stimulation by R light and inhibition by FR light. Burial of seeds to a 10-cm depth for 3 to 6 months induced dormancy with a R or natural light requirement for germination.

Palmer amaranth seeds developing under shade conditions (87% reduction in PAR) showed increased dormancy, a survival mechanism in low-light environment. In

addition, seeds maturing in the bottom-third of a mother plant exhibited increased dormancy, partially explaining variability in timing and extent of germination within a single seed population. Besides exhibiting increased seed dormancy, Palmer amaranth showed photosynthetic and morphological acclimation to 87% shading. These characteristics make Palmer amaranth a troublesome weed in crop-production systems.

Based on this research, an early-season glyphosate application preferably at the V3 stage of glyphosate-resistant soybean in conjunction with early planting dates (April) with narrow row (18-cm wide) spacing can be a promising strategy to reduce Palmer amaranth interference and seed production and improve soybean yields.

DEDICATION

From the core of my heart, I dedicate all the hard work and sincere efforts it took to complete my Ph.D. to my father Mr. Santosh Kumar Ojha, my mother Mrs. Narayani Ojha, my brother Mr. Sushant Jha, and my beloved wife Mrs. Anjani Jha. My father has been a positive force and motivation behind my successful achievement of this endeavor. I am indebted to my wife, mother, and brother for all their love, affection, sacrifice, and incessant encouragement. I am thankful to the almighty for giving me strength and blessings.

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CHAPTER 1

LITERATURE REVIEW

Palmer Amaranth

Palmer amaranth, a dioecious summer annual, is one of the most troublesome weeds of soybean in South Carolina (Norsworthy 2003) and is known to reduce crop yields and interfere with harvest (Bensch et al. 2003; Keeley and Thullen 1989; Klingaman and Oliver 1994; Massinga et al. 2001). Palmer amaranth is resistant to several herbicide families including imidazolinone, sulfonylurea, dinitroaniline, and triazine (Heap 2007). Glyphosate-resistant Palmer amaranth has been reported in several states in the southern United States (Culpepper et al. 2006; Mueller et al. 2006; Norsworthy et al. 2008; York et al. 2007). The competitiveness of this weed is due to its extended period of germination, relatively fast growth, prolific seed production (approximately 1 to 1.5 million seeds per plant) and long term seed viability (Horak and Loughin 2000; Sellers et al. 2003). Palmer amaranth produced the greatest leaf area, primary branches, and dry matter among four *Amaranthus* species, indicating its high level of competitiveness in a crop-weed environment (Horak and Loughin 2000).

Weed Seed Bank Dynamics and Seedling Emergence

Predicting weed seedling emergence is important for developing integrated weed management strategies (Cardina et al. 1996). This requires a thorough understanding of weed seed bank dynamics and behavior of weed seeds in the soil (Buhler et al. 2001).

The largest source for the soil seed bank is the plants that escape control and produce seeds within the field (Cavers 1983). The proportion of the weed seedlings that emerge from the soil seed bank varies among species and environmental conditions (Buhler et al. 2001). Additionally, emergence of weed seedlings depends on the initial weed density (Buhler 1999a), and the percent emergence is generally greater during the first growing season following introduction of seeds into the soil (Egley and Williams 1991).

The emergence of weeds from the soil seed bank also depends on the dormancy level of the seeds (Benech-Arnold et al. 2000). This is influenced by factors like soil temperature, soil moisture, and light availability. Temperature has been used to describe the emergence potential and timing of weed emergence for various species (Benech-Arnold et al. 2000; Bridges et al. 1989; Forcella 1993).

Crop and weed management practices influence changes in the soil weed seed population (Wilson 1988). The use of herbicides and other cultural practices like tillage significantly decrease the weed seeds entering the soil but cannot completely deplete the weed seed bank. Buhler (1999b) showed that weed free conditions for four years significantly reduced the weed seed numbers, but sufficient weed seeds remained in the soil to reduce crop yields during the fifth year. Burnside et al. (1986) observed a 95% decline in the weed seed density after a 5-year weed-free period, but during the subsequent year when herbicides were not used, there was a 90% increase in the weed seed density compared to the original seed bank.

The weed seed bank has also been studied in terms of vertical distribution of seeds

in the soil profile. Tillage is a major mechanism for vertical movement of weed seeds in soil (Buhler et al. 2001). Under intensive tillage systems like moldboard-plowing, weed seeds are evenly distributed throughout the upper 20 to 30 cm of the soil profile (Buhler et al. 2001; Wilson 1988). Buhler et al. (2001) concluded that under a no-tillage system, the majority of weed seeds are present in the upper 1 cm of the soil and very few are present below 10 cm. The downward movement of the weed seeds in the soil can also be influenced by the size of the seeds. For example, the small size of common waterhemp seeds facilitates its downward movement into the soil profile (Thompson et al. 1993).

Seed Dormancy and Germination

Seed dormancy is the inability of viable seeds to germinate. It is a survival mechanism for weeds and allows them to persist in the soil seed bank over extended periods (Baskin and Baskin 1985; Benech-Arnold et al. 2000). In general, seed dormancy has been categorized as primary or secondary dormancy. Primary dormancy is the state of arrested germination of mature, fully imbibed seeds (Foley 2001). Benech-Arnold et al. (2000) defined primary dormancy as the innate dormancy possessed by seeds after dispersal from the mother plant. Secondary dormancy refers to a dormant state induced in a non-dormant or primary dormant seed by unfavorable conditions for germination (Baskin and Baskin 1998).

Many weed species show annual changes in dormancy which are referred to as dormancy cycles (Baskin and Baskin 1985; Baskin and Baskin 1998). The non-dormant

seeds pass from a period of primary dormancy followed by a period of non-dormancy followed by a re-entry into dormancy (Benech-Arnold et al. 2000). These annual changes in the dormancy cycle are regulated by the interaction of environmental factors including temperature and light (Foley 2001; Leon and Owen 2003).

Effect of Maternal Environment

Seed dormancy is also established in the mother plant during seed maturation and is thus affected by the environmental conditions during seed development (El-Keblawy and Al-Ansari 2000; Gutterman 1992; Kigel et al. 1977). Photoperiod at the time of seed maturation affects seasonal changes in seed dormancy (Baskin and Baskin 1998; Munir et al. 2001). Photoperiod influenced germination response of Arabidopsis seeds to cold stratification (4 C for 21 d). The short-day maternal photoperiod enhanced germination of stratified seeds; however, it inhibited germination of non-stratified seeds. This suggests the effect of progeny stratification on seed dormancy and germination through the expression of a photoperiodic effect (Munir et al. 2001). Seeds of redroot pigweed grown under a short-day length had higher germination in response to cold stratification and short light exposures than those grown under long-day lengths (Kigel et al. 1977). Additionally, effect of maternal photoperiod on seed coat development of weed seeds have been reported (Gutterman 1992). Differences in seed coat thickness have been shown to affect seed dormancy due to differences in gas diffusion and imbibition (Stabell et al. 1998; Werker 1981).

Effect of Burial Depth

Buried weed seeds constitute an important part of the soil seed bank (Baskin and Baskin 1985, 1998; Benvenuti et al. 2001). Position and distribution of these seeds in soil play an important role in germination and subsequent emergence (Benvenuti and Macchia 1997; Burnside et al. 1981). *In-situ* germination of redroot pigweed seeds in the field showed a decline in germination with an increase in burial depth from 0 to 10 cm (Omami et al. 1999). The intact non-germinated seeds exhumed from the field showed a different response. At a temperature shift of 12 C in dark for 7 days followed by 35 C in light for 14 days, seeds lying on the soil surface had less germination compared with the seeds recovered from a 10-cm soil depth (Omami et al. 1999). Benvenuti et al. (2001) also reported a decrease in redroot pigweed seedling emergence with an increase in burial depth and the emergence was found to be less than 10% at a burial depth of 8 cm. Emergence of the buried seeds is also dependent on seed size. Some weed seeds like velvetleaf emerged even from a depth of 10 cm, although the emergence was only 5 to 15% (Benvenuti et al. 2001).

Duration of seed burial plays an important role in dormancy and germination of weed seeds (Baskin and Baskin 1985; 1998). Germination of redroot pigweed declined after 1 month of seed burial with the lowest for seeds buried for 3 months. Peak germination occurred after 9 months of burial and again declined at 12 months after burial (Omani et al. 1999). Omani et al. (1999) reported cyclic changes in dormancy and germination of redroot pigweed seeds during a 12-month burial period. The decrease in

germination with increase in burial depth of annual weed seeds is partially due to the induction of dormancy (Benvenuti et al. 2001; Milberg and Andersson 1997). Depth-mediated seasonal changes in dormancy and germination have been reported in buried seeds of annual weed species such as redroot pigweed and velvetleaf (Benvenuti et al. 2001; Milberg and Andersson 1997).

Milberg and Andersson (1997) reported that burial in soil induced a light requirement in some weed seeds. Burial induces light sensitivity in some weed seeds with a shift from low fluence response (LFR) to very low fluence (VLFR) response of the phytochrome (Gallagher and Cardina 1998a,b; Scopel et al.1991; Smith 1995). For example, seed burial induced VLFR germination in redroot pigweed and smooth pigweed (Gallagher and Cardina 1997), which may also be expected with Palmer amaranth.

The seasonal acquisition of dormancy in buried seeds is also affected by soil moisture and gaseous environment (Gallagher and Cardina 1997; Holm 1972; Benvenuti and Macchia 1997). Photoinduction of redroot pigweed by the conversion of Pr form of phytochrome to photosynthetically active Pfr form depends on the hydration of phytochrome (Gallagher and Cardina 1997). With an increase in burial depth, there is a decrease in oxygen concentration, leading to hypoxia and germination inhibition in seeds of some weed species (Holm 1972). Thus, burial depth plays an important role in mediating dormancy and germination of weed seeds in the soil seedbank.

Effect of Temperature

In most summer annuals, dormancy alleviation occurs through widening of the thermal range with a decrease in minimum temperature required for germination (Baskin and Baskin 1985; Benech-Arnold et al. 2000). All weed species have a minimum temperature requirement for germination, and some species respond positively to temperature fluctuations for germination and dormancy alleviation (Baskin and Baskin 1998). Leon et al. (2004) reported that dormancy of common waterhemp seeds was reduced largely in response to temperature fluctuations compared to constant temperature. Temperature fluctuations triggered germination of dark as well as far-red (FR) treated seeds of broadleaf dock and the effect was more pronounced for 10 C fluctuation (20 to 30 C) compared to 5 C fluctuation (22 to 27 C) (Benvenuti et al. 2001). High fluctuating temperatures can break seed coat-imposed physical dormancy of seeds (McKeon and Mott 1982).

In summer annual weeds, dormancy release is believed to occur at low temperatures during winter, while re-induction of dormancy occurs at high temperatures during early summer (Baskin and Baskin 1985; Benech-Arnold et al. 2000). Love-lies-bleeding (*Amaranthus caudatus*) seeds germinated at an optimum temperature range of 25 to 35 C, but exposure of seeds to 45 C inhibited germination due to induction of secondary dormancy (Kepczynski and Bihun 2002). Chilling alleviates secondary dormancy in most summer annuals (Baskin and Baskin 1985,1998). Development of VLFR sensitivity in redroot pigweed subjected to incubation temperatures of 5, 12, and

23 C was higher at 23 C, and induction of germination by the lowest fluence (3 µmol m^{-2}) was greater at 30 C than 20 C (Gallagher and Cardina 1998b).

Effect of Light

Light plays a significant role in dormancy and germination of many weed seeds. Light is required for dormancy breaking and germination of small-seeded weeds like *Amaranthus* species, which germinate only from shallow soil-depths of 0.5 to 2.5 cm (Buhler et al. 1996; Oryokot et al. 1997). Leon and Owen (2003) reported a phytochrome mediated seed dormancy in common waterhemp. Exposure to red (R) light induced germination in dormant seeds of common waterhemp, and this effect was more pronounced in chilled seeds compared to non-chilled seeds, suggesting an interaction of low temperatures with phytochrome in dormancy alleviation (Gallagher and Cardina 1997, 1998a,b). Low R/FR ratio inhibited germination in common waterhemp, suggesting that FR light induces dormancy (Leon and Owen 2003). This inhibitory effect of FR light is due to decrease in Pfr/Pr of the phytochrome, which may lead to photodormancy in seeds (Benech-Arnold et al. 2000; Frankland and Taylorson 1983).

Effect of Hormones

Gibberellic acid (GA) and ethylene are two important plant growth regulators which play a significant role in dormancy alleviation and germination of seeds. Kepczynski et al. (2003) demonstrated the involvement of ethylene in breaking of

primary dormancy in redroot pigweed seeds and found that the dormant seeds require more ethylene than non-dormant seeds for germination. GA sensitizes redroot pigweed seeds to VLF light and thus promotes germination in response to brief exposures to light (Gallagher and Cardina 1998b).

Abcisic acid (ABA) is an inhibitor of seed germination and plays an important role in embryo dormancy (Bewley 1997; Bewley and Black 1994). Kepczynski and Bihun (2002) found that the secondary dormant seeds of Love-lies-bleeding required a lower concentration of ABA than non-dormant seeds for germination inhibition, suggesting that the ABA concentrations are higher in dormant seeds. Decrease in ABA levels and increase in GA levels resulted in dormancy alleviation and seed germination in some weed species (Moore 1979).

Effect of Stratification

Stratification is the exposure of moist seeds to varying lengths of time at different cold and warm temperatures. It can influence the dormancy level and light sensitivity of seeds of several weed species like dandelion (Noronha et al. 1997). Leon and Owen (2003) found that stratification (moist chilling) of common waterhemp seed reduces dormancy and induces sensitivity to light for germination. After chilling at 4 C, germination of common waterhemp seeds was promoted by exposure to R light, whereas exposure to FR light inhibited germination and maintained dormancy. Chilling increases endogenous GA and decreases ABA, leading to dormancy alleviation in seeds of some

species (Moore 1979).

Effect of Scarification

Scarification is the process of breaking the hard, impermeable seed-coat which acts as a barrier for the entry of oxygen and moisture. Seed coat imposed physical dormancy has been reported in 15 plant families including Convolvulaceae (morningglory family) (Baskin et al. 2000). It has been reported that acid or mechanical scarification can break the seed coat imposed dormancy and induce germination. Acid scarification dissolved the outer palisade cells of the hilium in blue lupin (*Lupinus angustifolia*) seeds and increased water imbibition of the seeds (Burns 1959). Mechanical or chemical scarification with 10 M sulphuric acid for 1 to 5 minutes resulted in germination of the secondary dormant Love-lies-bleeding seeds at 25 C (Kepczynski and Bihun 2002).

Physiological Effects of Shading on Survival and Growth

Shading affects the survival and growth of plants by altering their physiological and morphological response to light environment. Shading causes reduction in photosynthetic photon flux density (PPFD) and R/FR light perceived by the weeds beneath the canopy (Norsworthy 2004; Thompson and Grime 1983). Increasing shade levels reduced growth and development and subsequent seed production of velvetleaf (Bello et al.1995). Velvetleaf plants grown in 30 and 76% shade had less height, leaf and branch number, and dry weight compared to those grown in the absence of shade.

Increased shading also reduced the number of velvetleaf capsules and seeds (Bello et al. 1995).

Holt (1995) concluded that weeds show plasticity in their response to shade. Shaded leaves transpire less than the leaves exposed to direct sunlight (Pons et al. 2001). Velvetleaf and tumble pigweed plants responded to shade by decreasing light saturated photosynthesis, dark respiration rates, and leaf thickness, while increasing chlorophyll content per unit leaf volume and specific leaf area (Patterson et al. 1978; Stoller and Myers 1989). Decrease in light compensation point and dark respiration are shade acclimation mechanisms of plants that allow them to maintain a positive carbon balance under low-light environment (Stoller and Myers 1989).

Weed Control

Effect of Glyphosate Timing

Glyphosate-resistant technology has gained popularity among soybean farmers throughout the United States. Glyphosate can be applied over-the-top of glyphosate-resistant soybean for postemergence control of weeds. Van Acker et al. (1993) determined the critical weed-free period for soybean to be from emergence to the V2 to V3 growth stage. Malugeta and Boerboom (2000) found that weed control efficacy and soybean yields were more influenced by glyphosate application timing than the rate, and applications at V2, V4, and R1 stages gave complete control of weeds.

Palmer amaranth and redroot pigweed emerging with soybean causes greater yield

losses than later emerging weeds at similar densities. At a density of 2 plants m^{-1} of row, Palmer amaranth and redroot pigweed that emerge at VE stage of soybean resulted in 12% yield loss compared to 0% for those that emerge at the V2 growth stage (Dieleman et al. 1995). Hartzler et al. (2004) reported that 90% of the common waterhemp plants that emerge at the time of soybean emergence survived, whereas the survival was 13% for those that emerge 50 days after planting. Additionally, common waterhemp biomass and seed production was less for the later emerging cohorts.

A single application of glyphosate does not provide season long control of difficult-to-control (tolerant) weed species; therefore, sequential applications are needed for complete control and higher crop yields (Norsworthy et al. 2001; Norsworthy and Oliver 2002; Payne and Oliver 2000). Single or sequential applications of glyphosate provided 100% control of Palmer amaranth 9 WAE, but a single application resulted in less control of entireleaf morningglory than sequential applications (Norsworthy 2004). Sequential applications of glyphosate in glyphosate-resistant soybean (18- to 51-cm width rows) improved yields and net returns (Reddy and Whiting 2000). Sequential glyphosate applications may be needed to control the replenishment of the soil seed bank and late season interference from the weeds that escape or survive an initial application.

Effect of Row Spacing

Soybean row spacing plays an important role in emergence and survival of weeds, and soybean yields. Complete canopy closure (95% or greater light interception) occurs 3

weeks earlier in a 30- compared to a 91-cm width soybean row (Murdock et al. 1986). Due to early-season canopy closure, there is increase in soybean leaf area index (LAI), leading to greater competition and weed suppression ability in narrow-row soybean (Howe and Oliver 1987; Norsworthy and Oliver 2002). Norsworthy (2004) reported that common cocklebur and sicklepod emergence was diminished after soybean canopy formation due to decrease in daily diurnal soil temperature fluctuations and increase in light interception by the canopy.

The light quality (R/FR) differences beneath the canopy may have also led to decline in weed emergence (Ghersa et al. 1994; Norsworthy and Oliveira 2007; Thompson and Grime 1983). This effect is more pronounced in narrow- compared to wide-row soybean. Rapid canopy closure of 18-cm narrow-row glyphosate-resistant soybean allowed greater flexibility in glyphosate application, reduced weed emergence, and late-season interference in comparison to 76-cm wide-rows (Malugeta and Boerboom 2000). The overall objectives of this research were to study 1) tillage and soybean canopy effects on Palmer amaranth emergence, 2) temperature and light requirements for germination of Palmer amaranth over a 12-mo period following seed maturation, 3) effects of maternal shading and seed location the mother plant on Palmer amaranth seed dormancy, 4) photosynthetic and morphological acclimation of Palmer amaranth to shading, and 5) influence of row-width and glyphosate timing on Palmer amaranth demographics in glyphosate-resistant soybean.

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CHAPTER 2

SOYBEAN CANOPY AND TILLAGE EFFECTS ON EMERGENCE OF PALMER AMARANTH FROM A NATURAL SEED BANK

Abstract

Effects of soybean canopy and tillage on emergence of Palmer amaranth were studied in 2004 and 2005 at sites with a natural infestation of Palmer amaranth at Pendleton, SC. In 2006, the effect of presence or absence of soybean was validated only in no-tillage plots. Palmer amaranth emerged from May 10 through October 23, May 13 through September 2, and April 28 through August 25 in 2004, 2005, and 2006, respectively. Two to three well-defined emergence periods occurred from early May through mid-July. Increases in light interception following soybean canopy formation were evident by early July, resulting in reduced Palmer amaranth emergence beneath the canopy, especially in no-tillage conditions. Of the total emergence during a season, >90% occurred prior to soybean canopy closure. Shallow spring tillage had minimal influence on Palmer amaranth emergence. Emergence was largely dependent on soil temperature and rainfall conditions each year.

Introduction

Palmer amaranth (*Amaranthus palmeri* S. Wats.) is a dioecious summer annual belonging to the Amaranthaceae (pigweed) family (Fernald 1950). It is one of the most problematic weeds of cotton, corn, and soybean in the southern United States (Kelley and Thullen 1989; Klingman and Oliver 1994; Massinga et al. 2001; Webster and MacDonald 2001). The success of Palmer amaranth as a major weed in crops is attributed to its rapid growth owing to a C_4 photosynthetic pathway (Ehleringer 1983; Jha et al. 2008a), prolific seed production (Keeley et al. 1987), and resistance to multiple herbicide chemistries, including glyphosate (Culpepper et al. 2006; Gossett et al. 1992; Horak and Peterson 1995).

Understanding seedling emergence is critical to improving weed management strategies (Buhler et al. 1998; Forcella et al. 1992, 2000; Myers et al. 2004). This includes determining the effect of tillage and crop canopy formation on seedling emergence. Soil temperature is often lower and soil moisture is higher in reduced tillage compared with conventional tillage systems (Addae et al.1991; Leon and Owen 2006). Subtle differences in the soil microclimate may have large affects on seedling emergence of weeds including *Amaranthus* species (Buhler et al. 1996; Mohler 1993; Oryokot et al. 1997). Additionally in a no-tillage system, seeds are concentrated in the upper 5 cm of the soil profile relative to conventional tillage systems (Buhler 1992; Cardina et al. 1991; Clements et al. 1996), which allows small-seeded weeds such as pigweeds to emerge

more easily from shallow depths (Buhler et al. 1996; Oryokot et al. 1997; Webb et al. 1987).

Besides tillage, crop canopy can have a suppressive effect on weed seedling emergence, as it dampens the soil thermal amplitude and alters the light quality perceived by seeds lying on the soil surface (Batlla et al. 2000; Fortin and Pierce 1990; Norsworthy 2004). A reduction in the red:far-red (R:FR) ratio as a result of increased FR transmitted light beneath a crop canopy has been well documented (Norsworthy 2004; Sattin et al. 1994; Thompson and Grime 1983). This phenomenon causes inhibition of seed germination in some weed species including *Amaranthus* species like redroot pigweed (*Amaranthus retroflexus* L.), smooth pigweed (*Amaranthus hybridus* L.), and common waterhemp (*Amaranthus rudis* Sauer.) (Fenner 1980; Gallagher and Cardina 1998; Leon and Owen 2003; Taylorson and Borthwick 1969). Crop canopy effects on reduced emergence have been previously reported in weeds such as common cocklebur (*Xanthium strumarium* L.) and sicklepod (*Senna obtusifolia* L.) (Norsworthy 2004).

Effects of crop canopy and tillage on emergence characteristics of *Amaranthus* species such as redroot pigweed and common waterhemp has been previously studied (Anderson and Nielsen 1996; Cardina et al. 2002; Hartzler et al. 1999; Leon and Owen 2006; Oryokot et al. 1997). However, research on the emergence pattern of Palmer amaranth is lacking. The objectives of this study were to quantify the effects of tillage and soybean canopy formation on emergence of Palmer amaranth from a natural seed bank.

Materials and Methods

Experiments were conducted at the Simpson Research Station near Pendleton, SC, in 2004, 2005, and 2006 on sites with a natural infestation of Palmer amaranth. The soil was a Cecil sandy-loam (fine, kaolinitic, thermic Typic kanhapludults). Experiments were designed as randomized complete block with a factorial arrangement of spring tillage and no spring tillage with and without soybean with four replications of each treatment. In 2006, emergence was monitored only in no-till plots with or without soybean. Tillage plots were disk harrowed once on April 18, 2004, and April 22, 2005, to a depth of 10 to 15 cm and then cultivated once with a rotary-tiller on May 19, 2004 and May 18, 2005 to a depth of 10 cm.

Maturity group VI glyphosate-resistant soybean cultivars 'Asgrow 6202' and 'Delta and Pine Land 6880' were planted on May 21, 2004 and May 18, 2005, respectively. In 2006, an early maturing Group IV soybean cultivar 'Delta and Pine Land 5915' was planted on April 12. Soybean seeds were planted at a 2.5-cm soil depth in 18-cm rows at a density of 432,000 seeds ha^{-1}. Soybean plants were removed from the 'no soybean' plots by clipping plants at the soil surface following emergence.

The emergence of Palmer amaranth seedlings was monitored in 1 m^2 plots from mid-March through November each year. Emerged seedlings were identified, counted, and removed by hand three times a week from April to August and at least once a week during March, September, and October, depending on the density of emergence. Once a month following Palmer amaranth emergence counts, glyphosate at 0.84 kg ae ha^{-1} was

applied using a hand-held boom at 5 km h^{-1} calibrated to deliver 94 L ha^{-1} at 276 kPa. Glyphosate was applied to prevent infestation of the test site with other weeds that could interfere with Palmer amaranth emergence or with soybean establishment.

Soil temperature at a 2.5-cm soil depth was recorded hourly from April through October of each year using data loggers installed in two to three plots per treatment. Soil temperature data were used to estimate daily minimum and maximum temperatures during a season (Figures 2.1, 2.2, and 2.3). Daily rainfall data (Figure 2.4) were collected from a weather station located approximately 1.5 km from the experimental site. Following soybean emergence, photosynthetically active radiation (PAR, mol m^{-2} s^{-1}) was measured at least once a week within 1.5 h of solar noon using a line quantum sensor in plots with soybean. Percent light interception by soybean was calculated using Equation 1,

$$\%LI = (a - b)/a \times 100 \quad [1]$$

where *LI* represents percent light interception by soybean, *a* is PAR measured above the soybean canopy, and *b* is PAR at the soil surface beneath the canopy. The light level at the soil surface (*b*) was the average of two random readings from each measured plot, taken perpendicular to the planted rows.

Data collected at the sampled dates were used to calculate cumulative and daily emergence per square meter. Cumulative emergence was calculated as the sum of emergence on a sample date and the previously sampled date. Daily emergence was

calculated by dividing the emergence counts on a sample date with the number of days between a sample date and the previously sampled date. A "quality control approach" developed by Montgomery et al. (2001) and used in previous studies (Norsworthy and Oliveira 2007) was used to determine the peak emergence periods. A peak emergence was considered to occur when the daily emergence was greater than the total number of emerged seedlings during the season divided by the total emergence duration (daily emergence rate) plus the standard deviation of the daily emergence for a treatment. Data for sampled and cumulative emergence per square meter were log transformed [$\log_{10}$ (x+10)] to meet normality assumption, which was tested using the Shapiro-Wilk test. A factorial analysis of variance (ANOVA) was used to test the main effects of tillage and presence or absence of soybean on Palmer amaranth emergence. The analysis was achieved using PROC GLM in SAS. In addition, cumulative emergence for each sample date was subjected to ANOVA to identify the periods when tillage or soybean canopy affected Palmer amaranth emergence.

Results and Discussion

Palmer Amaranth Emergence Pattern

Data for Palmer amaranth emergence were presented by year due to inconsistencies in sampling dates, test sites, and environmental conditions between years (2004, 2005, and 2006). During 2004, Palmer amaranth emerged from May 10 through October 23, with three peak emergence periods from mid-May through mid-July (Figure 2.7). In 2005, emergence occurred from May 13 through September 2, with two to three peak periods from early May through early June (Figure 2.8). In 2006, Palmer amaranth emerged from April 28 through August 25, with a minor peak occurring in late May and a major one occurring in late June (Figure 2.9).

The timing and occurrence of major emergence flushes coincided with soil temperature and moisture conditions during a year. One wk prior to Palmer amaranth emergence, the average minimum and maximum soil temperatures were 20 and 35 C, respectively, in 2004 (Figure 2.1); and 17.5 and 35 C, respectively, in 2005 (Figure 2.2). Mean temperature for germination of Palmer amaranth is ≥25 C (Guo and Al-Katib 2003; Wright et al. 1999; Jha et al. 2008b). In 2006, a daily mean temperature of 25 C was attained in late April (Figure 2.3), resulting in an earlier emergence than the other two years. Timing of emergence peaks was largely contingent upon rainfall each year. From March through early July, there were only five precipitation events >10 mm in 2004 compared with fifteen precipitation events >10 mm in 2005 (Figure 2.4). Two major rainfall events occurred in May and June of 2006 (Figure 2.4) resulting in two emergence

flushes. Thus, high soil moisture and high temperatures favoring germination of pigweed seeds (Guo and Al-Katib 2003; Wright et al. 1999; Hartzler et al. 1999) were achieved during May to June, which allowed Palmer amaranth seedling recruitment over a shorter period with higher magnitudes of emergence (number of emerged seedlings m^{-2}) in 2005 and 2006.

A relatively dry spring of 2004 was followed by a period of rainfall. Precipitation from mid-July through September was 420 mm (Figure 2.4), which was greater than the 30-year average for that period at the test site in South Carolina. These conditions caused the non-dormant portion of the seedbank to germinate and emerge over a longer duration in 2004 than in any other year, with emergence peaks being relatively smaller in magnitude than in 2005 and 2006.

The effect of low rainfall on delayed pigweed emergence has previously ben reported (Hartzler et al. 1999; Oryokot et al. 1997). Redroot pigweed emergence was sensitive to small changes in rainfall (<1 mm) during the early growing season (Forcella et al. 1992). Besides high temperatures (≥25 C), low rainfall during spring (April and May) affects the timing and density of emergence of several other weed species including wolly cupgrass [*Eriochloa villosa* (Thunb.) Kunth], velvetleaf (*Abutilon theophrasti* Medik.), giant foxtail (*Setaria faberi* Herrm.), and common waterhemp (Hartzler et al. 1999).

Effect of Tillage

Total emergence of Palmer amaranth was not influenced by spring tillage in 2004 and 2005. The effect of tillage was evident only in 2005 at the beginning of the season (first two to three sampling dates following each tillage event). Stimulation of germination and subsequent emergence following tillage was possibly due to increased soil aeration, improved soil-seed contact, and elevated soil temperatures. Previous research has also shown similar effects of tillage on the soil microclimate (Norsworthy and Oliveira 2007; Litch and Al-Kaisi 2005).

Several studies have shown that soil temperature is lower and soil moisture is higher in the absence of tillage than in tilled systems (Addae et al. 1991; Blevins and Frye 1993; Leon and Owen 2006). In those studies, the soil moisture and temperature were measured at 5- to 10-cm or greater soil depths. In the present study, soil temperatures measured at a 2.5-cm depth did not vary significantly between spring tillage and no spring tillage plots, which was expected considering the rapid transfer of heat and moisture at a shallow soil depth. Small-seeded weeds such as pigweeds can germinate and emerge only from shallow burial depths (0.5 to 2.5 cm) (Buhler et al. 1996; Ghorbani et al. 1999; Oryokot et al. 1997). Thus, in the present study, Palmer amaranth seeds located below a 2.5-cm depth in the soil seed bank were not likely to emerge, irrespective of tillage. Additionally, shallow spring tillage does not appear to be an important factor in impacting seedling emergence which is similar to other *Amaranthus* species including redroot pigweed (Cardina et al. 2002; Oryokot et al. 1997).

Effect of Soybean Canopy

Percent light interception by soybean increased sigmoidally from soybean emergence through senescence (Figure 2.5). In 2004, a significant effect of soybean canopy on cumulative emergence of Palmer amaranth was first observed on July 9 (32 d after soybean emergence), when light interception by soybean was 75%. In addition, the interaction of soybean and tillage was significant ($P = 0.035$). The soybean canopy effect on emergence was more pronounced without than with spring tillage. Without spring tillage, a total of 102 seedlings m^{-2} were present on July 9 in the absence of soybean compared with 27 seedlings m^{-2} with soybean, a 73% decrease. In contrast, emergence during the same period in spring tillage plots with and without soybean was 11 and 59 seedlings m^{-2}, respectively, with the difference not being statistically significant.

Apart from reductions in PAR, soybean canopy closure caused reductions in soil thermal amplitudes (Figures 2.1, 2.2, and 2.3); however, the differences were consistent across tillage treatments. From July 9 through the first week of September (soybean senescence) in 2004, light interception by soybean increased from 75 to 90% (Figure 2.5). Over the same period, average daily soil temperature fluctuation at a 2.5-cm soil depth was 16.0 C in plots with soybean compared with 4.8 C in plots without soybean. High temperatures ($\geq$25 C mean) and thermal amplitudes ($\geq$7.5 C) favor germination of Palmer amaranth and other pigweeds (Gou and Al-Khatib 2003; Jha et al. 2008c; Steckel et al. 2004; Wright et al. 1999).

The inconsistency in the canopy effect across tillage treatments in 2004, although not clearly understood, might be related to differences in light availability of seeds with and without spring tillage. It is known that light penetration through soil is limited to a maximum depth of 4 mm (Benvenuti 1995). In this study, assuming that most Palmer amaranth emergence occurred within the upper 2.5-cm depth of soil, the number of seeds on or near the soil surface (light-transmittance zone) should have been greater in the absence of spring tillage. Hence, changes in the light environment at the soil surface as the canopy developed was expected to have a greater influence on germination of seeds in plots that did not receive spring tillage. Under a canopy, besides reductions in PAR, seeds experience an increase in far-red (FR) transmitted light (Norsworthy 2004; Taylorson and Borthwick 1969; Thompson and Grime 1983), which is inhibitory to germination of *Amaranthus* species including Palmer amaranth, which exhibit a phytochrome-controlled germination response (Gallagher and Cardina 1998; Hartzler et al. 1999; Jha et al. 2008c; Leon and Owen 2003).

Earlier soybean planting in 2006 allowed the crop to produce a dense canopy prior to the culmination of Palmer amaranth emergence, leading to reduced emergence in soybean plots. The effect of soybean on Palmer amaranth emergence was first significant on June 30, 33 days after soybean emergence, when the light interception in plots with soybean was 81% (Figure 2.5). From June 30 to August 25 (last day of Palmer amaranth emergence during the season), a total of 314 seedlings m^{-2} emerged without soybean compared with 75 seedlings m^{-2} with soybean, a 76% reduction. Over the same period,

average daily soil temperature fluctuation was 10.1 C without soybean compared to 5.1 C with soybean.

In 2005, light interception by soybean reached 75% on July 10 (Figure 2.5); however, there was no significant effect of presence or absence of soybean on Palmer amaranth emergence. It is difficult to explain the difference in results in 2005 compared to 2004 and 2006. Yearly variations in weed seedling emergence from natural soil seed banks could occur due to differences in weather conditions, experimental site, cropping history (crop rotations and tillage), seed source, and genetic factors (Cardina et al. 2002; Forcella et al. 1992; Hartzler et al.1999; Mohler 1993; Oryokot et al. 1997; Swanton and Murphy 1996). Variability in spatial distribution of weed seeds in the soil seed bank and higher variance in the magnitude of emergence within a population could also be contributing factors (Egley and Williams 1991; Malugeta and Stoltenberg 1997; Stoller and Wax 1973).

Results from the present study suggest that most emergence of Palmer amaranth from a natural seed bank in South Carolina occurred from early May through mid-July. The peak emergence periods during a season were concomitant with the periods having high mean temperatures and rainfall. The majority of the emergence also coincided with the recommended planting dates for soybean and cotton, which explains why this weed is among the most common and troublesome weeds of soybean and cotton in southeastern United States. Corn is generally planted a minimum of four weeks before initial Palmer amaranth emergence; thus, explaining why Palmer amaranth is less of a problem in corn.

In all years, more than 90% of the total emergence during a season occurred prior to the period (late June to early July) when a soybean canopy effect was first evident. This agrees with the results from our previous study showing that emergence of Palmer amaranth cohorts following soybean canopy closure (V6 stage) was minimal (Jha et al. 2008b), although emergence in the absence of soybean was not quantified in that study. It was also concluded that shallow spring tillage has minimal influence on cumulative emergence of Palmer amaranth. Based on the results from this study, early to mid-season (early May to late June) herbicide applications with an early crop canopy closure would be a promising strategy to manage Palmer amaranth.

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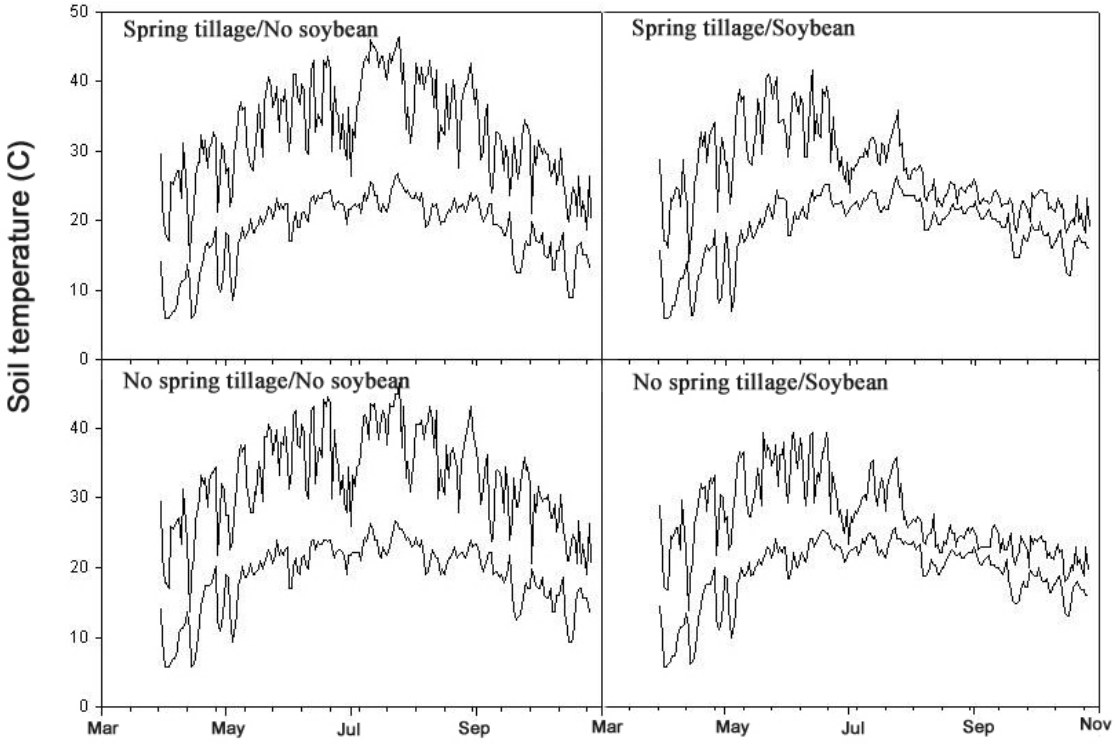

Figure 2.1. Daily maximum and minimum soil temperatures at 2.5-cm soil depth in 2004 at Pendleton, SC.

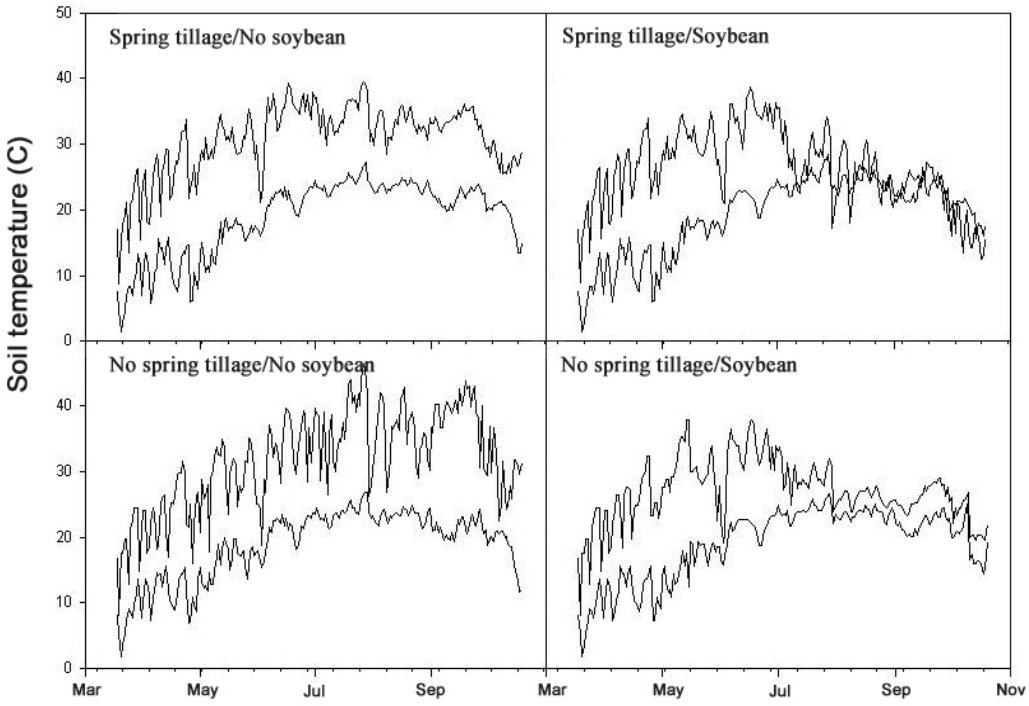

Figure 2.2. Daily maximum and minimum soil temperatures at 2.5-cm soil depth in 2005 at Pendleton, SC.

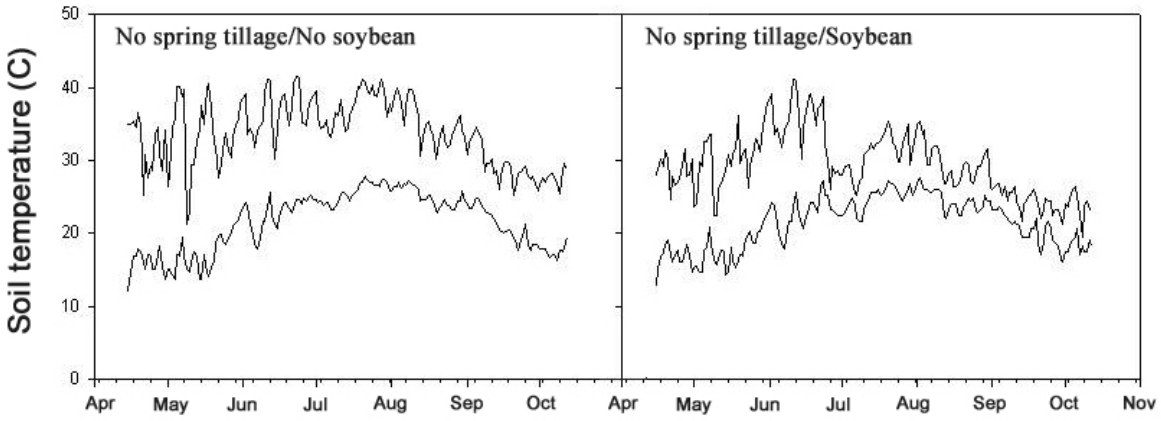

Figure 2.3. Daily maximum and minimum soil temperatures at 2.5-cm soil depth in 2006 at Pendleton, SC.

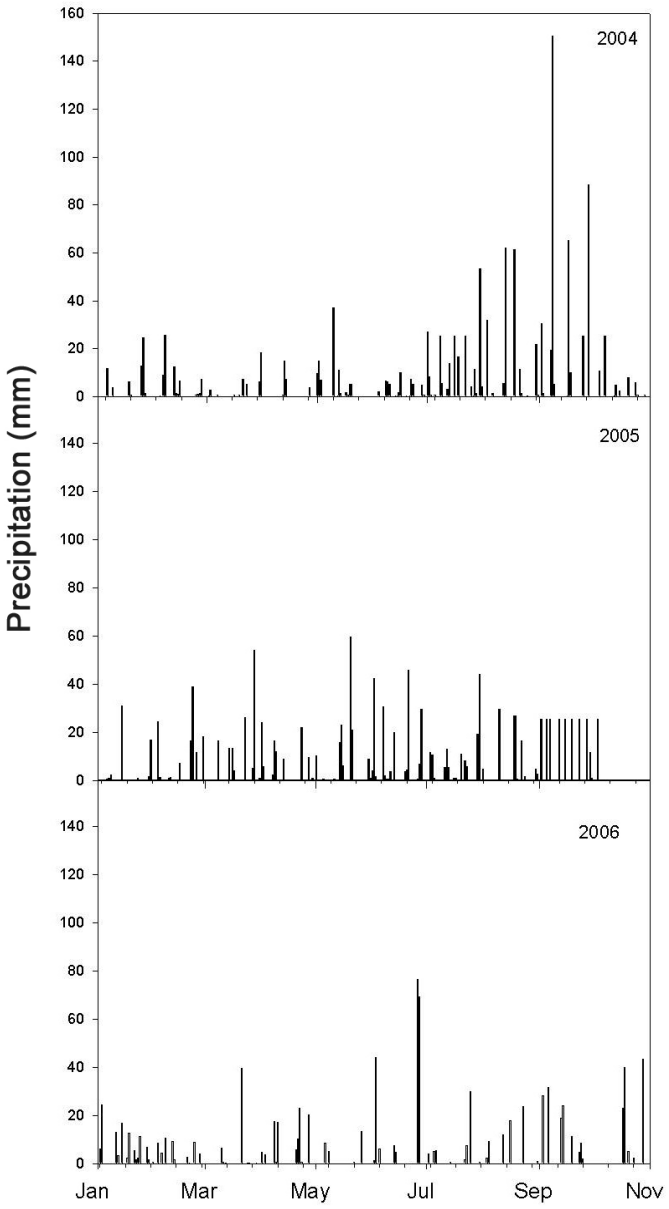

Figure 2.4. Daily precipitation in 2004, 2005, and 2006 at Pendleton, SC.

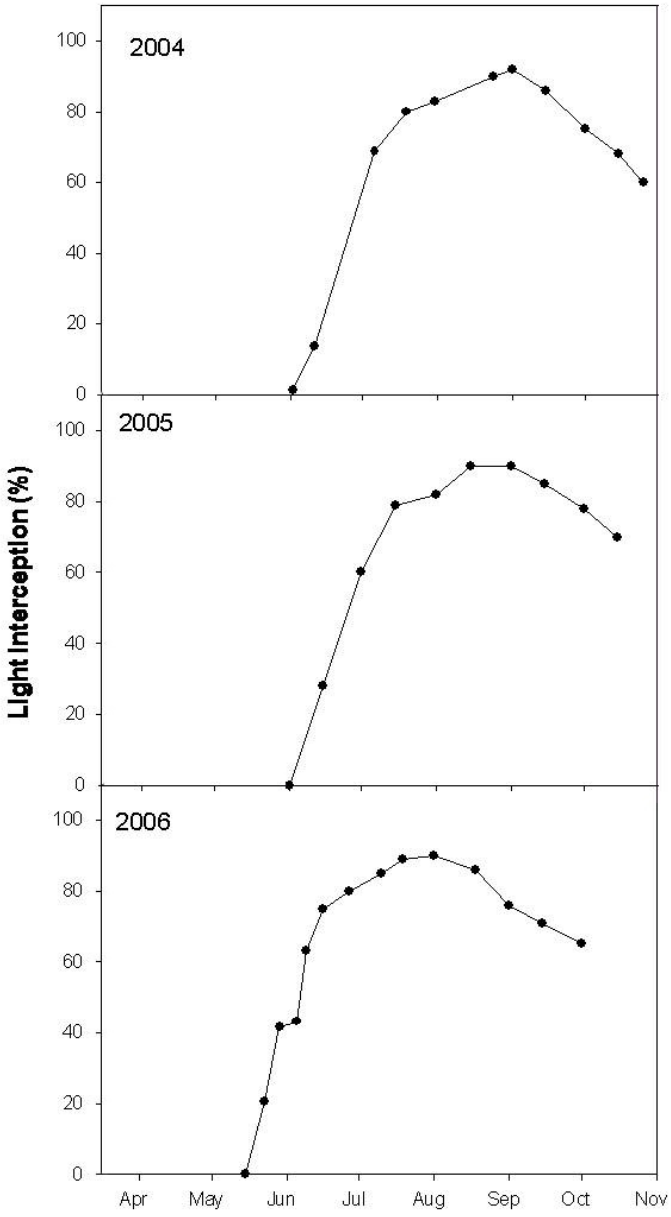

Figure 2.5. Percentage light interception by the soybean canopy in 2004, 2005, and 2006.

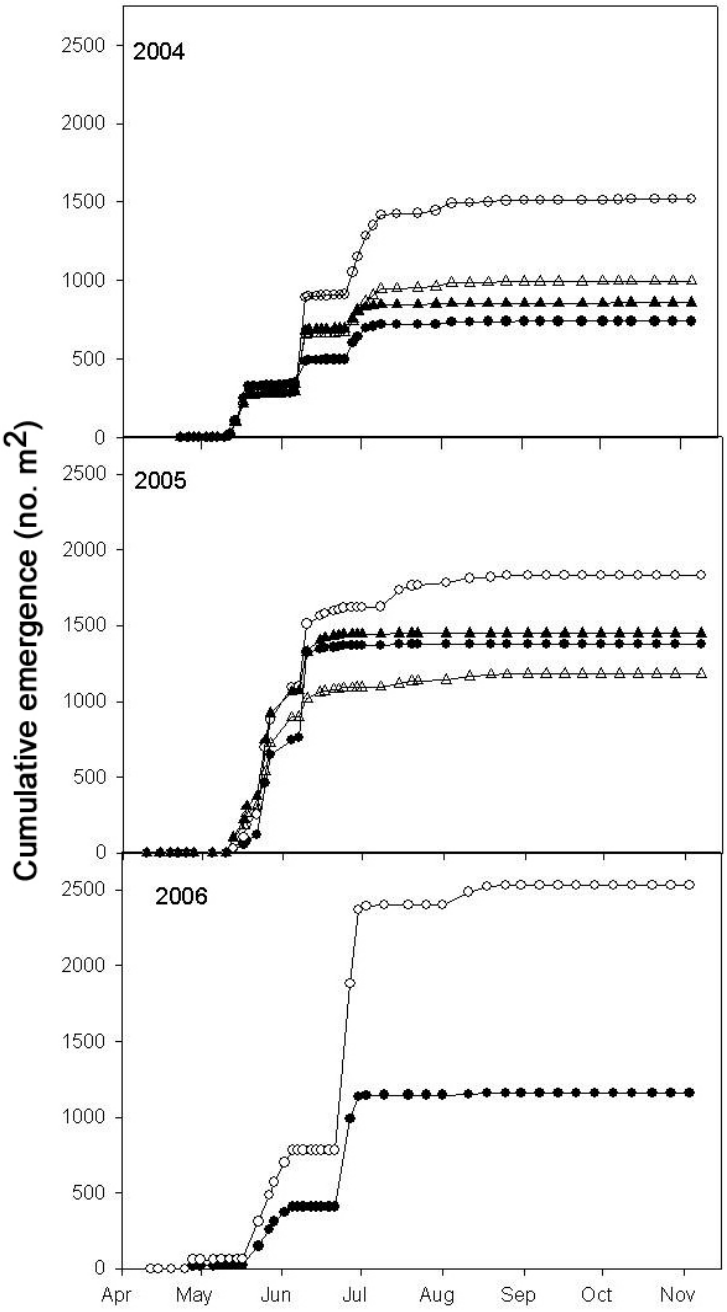

Figure 2.6. Cumulative emergence of Palmer amaranth in spring tillage and no-tillage plots in the presence and absence of soybean in 2004 and 2005 and in no-tillage plots with and without soybean in 2006.

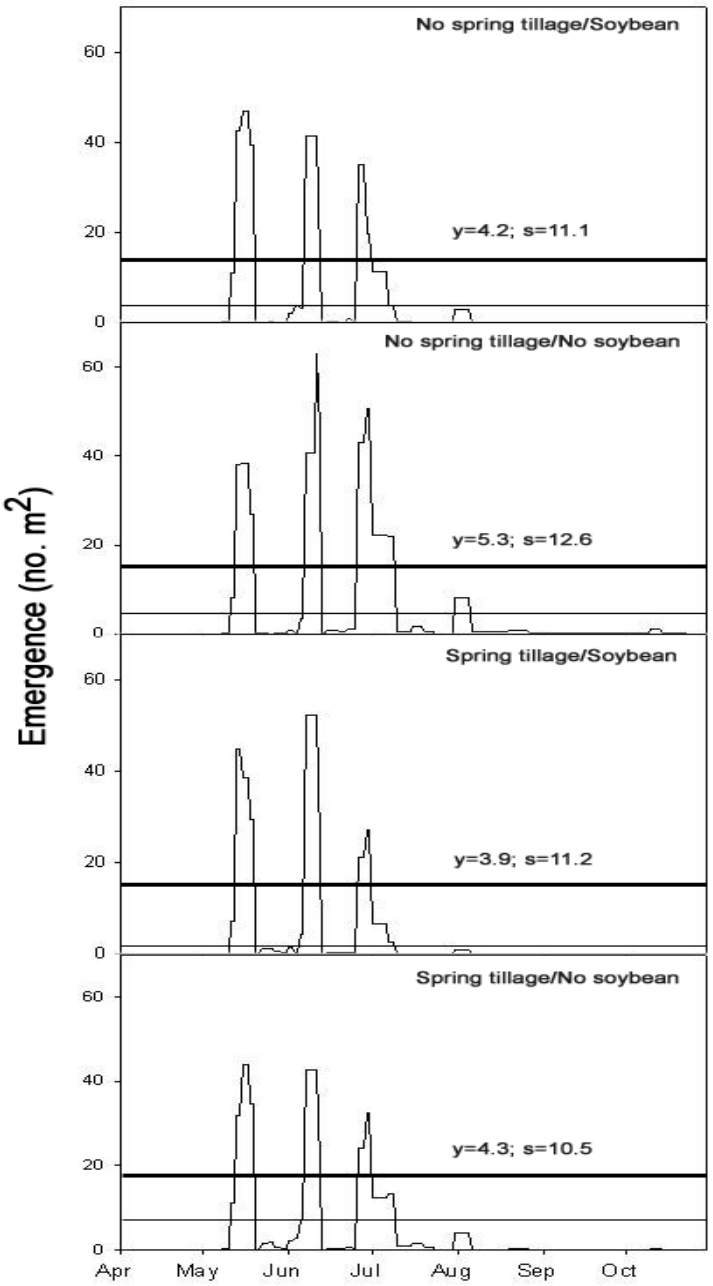

Figure 2.7. Daily emergence of Palmer amaranth in tillage and no-tillage plots with and without soybean in 2004. The thin solid horizontal line within each graph represents the daily mean emergence for a treatment (y); thick line represents the mean plus the standard deviation of the population for a treatment (s).

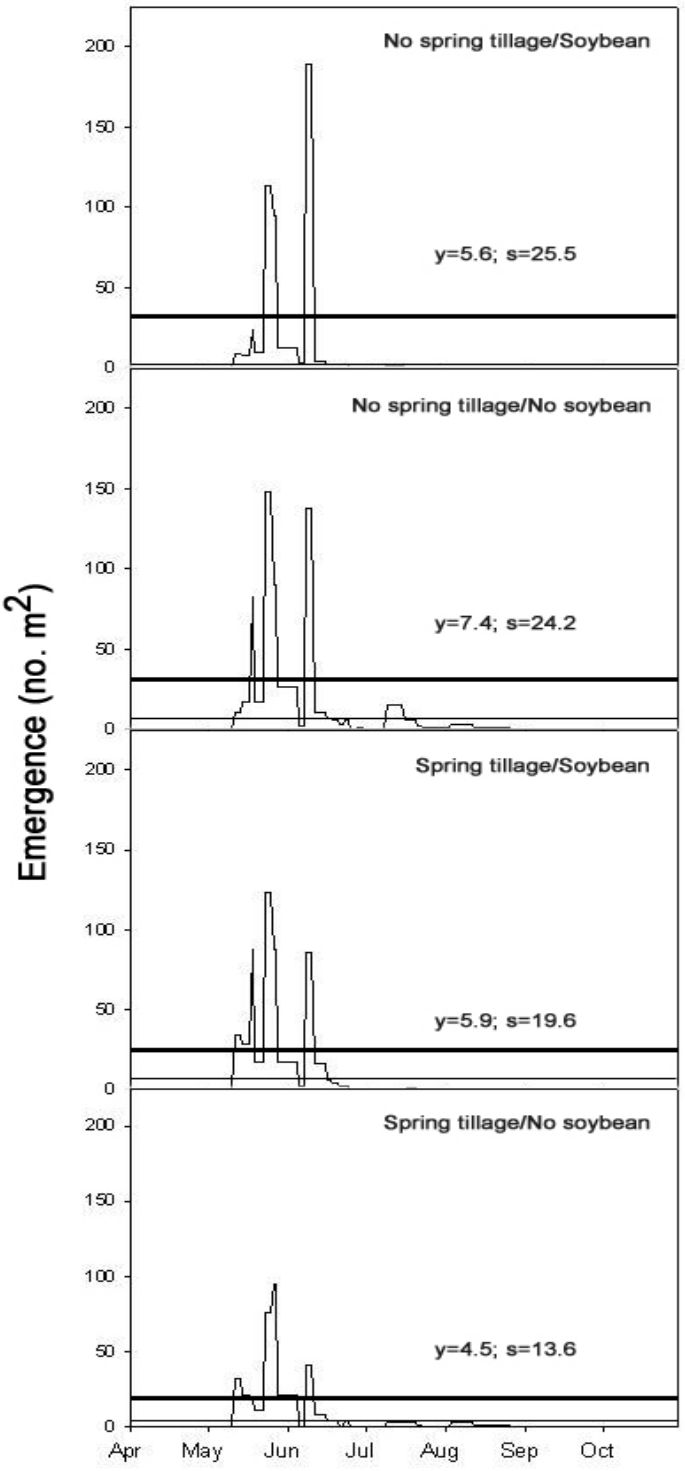

Figure 2.8. Daily emergence of Palmer amaranth in tillage and no-tillage plots with and without soybean in 2005. The thin solid horizontal line within each graph represents the daily mean emergence for a treatment (y); thick line represents the mean plus the standard deviation of the population for a treatment (s)

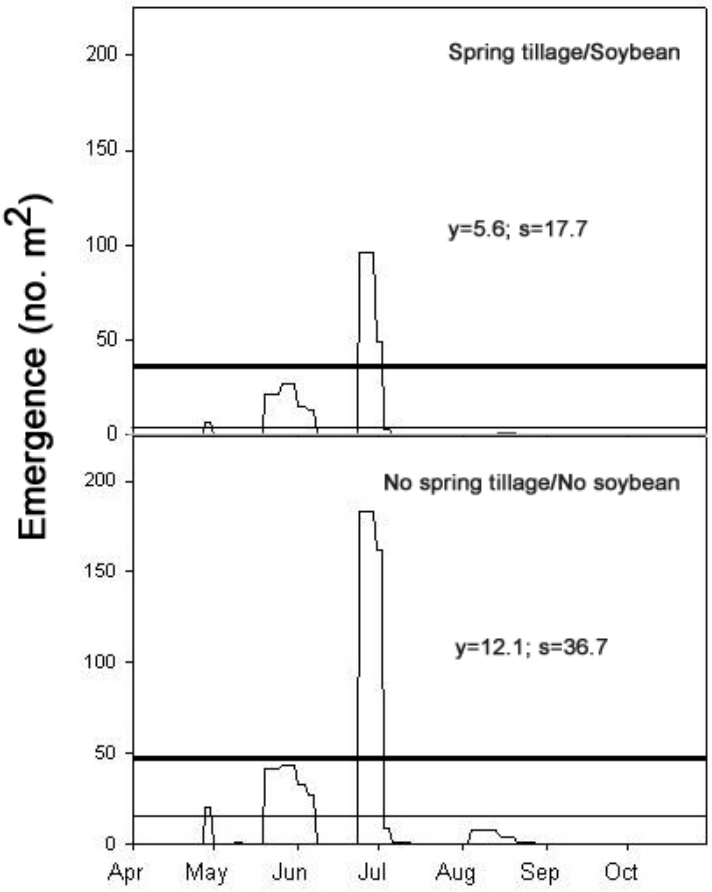

Figure 2.9. Daily emergence of Palmer amaranth in no-tillage plots with and without soybean in 2006. The thin solid horizontal line within each graph represents the daily mean emergence for a treatment (y); thick line represents the mean plus the standard deviation of the population for a treatment (s).

CHAPTER 3

TEMPERATURE AND LIGHT REQUIREMENTS FOR PALMER AMARANTH SEED GERMINATION OVER A 12-MONTH PERIOD

Abstract

Experiments were conducted on seeds collected in 2004 and 2006 from a natural population of Palmer amaranth near Pendleton, SC, to determine the temperature and light requirements for germination of seeds on the soil surface or buried over a 12-month period in the field. Germination of seeds in response to temperature and light varied over the 12-month period. Freshly matured seeds collected in November required constant and fluctuating temperature regimes of 25 to 40 C and 17.5/32.5 to 27.5/42.5 C, respectively, and natural or red (R) light for increased germination. Following after-ripening in the winter, seeds experienced a reduction in dormancy in February and displayed an average of 30% higher germination at mean temperatures ranging from 25 to 35 C. With after-ripening for an additional 3 months, seeds had the ability to germinate to a similar percentage across all evaluated constant and fluctuating temperatures; however, germination in natural light or R light was more than twice that in the absence of light and far-red (FR) light inhibited germination. Fluctuating temperatures improved germination during the after-ripening period, except in summer and fall (9- and 12-mo after maturation). Germination by fall was at least 50% higher in seeds retrieved from the soil surface when compared to those exhumed from a 10-cm depth, implying that burial for 3 to 6 mo induced dormancy in seeds. In addition, exposure of seeds to high after-

ripening temperatures during the summer caused secondary dormancy induction. Averaged across temperature amplitudes, seeds had higher germination at 25 to 40 C. After-ripened seeds required exposure to R or natural light for higher germination in fall, whereas, exposures to FR light or darkness inhibited germination. The effect of R and FR light was reversible, indicating Palmer amaranth germination was at least partially a phytochrome-mediated response.

Introduction

Seed germination under field conditions is mediated by seasonal changes in dormancy, which affects weed emergence patterns (Baskin and Baskin 1977, 1987, 1990; Honek et al. 1999; Omami et al. 1999). The annual dormancy cycling of weed seeds is known to be regulated by environmental factors such as temperature and light, implying that seeds in the soil seedbank undergo changes in their germination in response to temperature and light over time (Baskin and Baskin 1977, 1990; Benech-Arnold et al. 1988, 1990; Gallagher and Cardina 1998a,b; Leon et al. 2007; Norsworthy and Oliveira 2007). These changes prevent seed germination when conditions are unfavorable for growth and reproduction and ensure survival and persistence of weed seeds in the soil seedbank (Baskin and Baskin 1998).

In most summer annuals, after-ripening of seeds at low temperatures during winter causes dormancy release, while exposure to high temperatures during summer results in a re-induction of dormancy (Baskin and Baskin 1977, 1985b, 1987; Benech-Arnold et al. 2000). With dormancy alleviation, seeds experience a widening of the temperature range and a decrease in minimum temperature required for germination (Baskin and Baskin 1998; Benech-Arnold et al. 2000; Karssen 1982). *Amaranthus* species such as redroot pigweed (*Amaranthus retroflexus* L.) germinated at 10 C, but the optimal temperature for maximum germination of redroot pigweed, Palmer amaranth, and common waterhemp (*Amaranthus rudis* Sauer.) was from 25 to 35 C (Baskin and Baskin

1977; Ghorbani et al. 1999; Guo and Al-Khatib 2003; Kepczynski and Bihun 2002; Wright et al. 1999).

Following after-ripening during winter, seeds of most summer annuals require fluctuating temperatures for dormancy alleviation in spring (Baskn and Baskin 1977, 1987; Benech-Arnold et al. 1990). For instance, germination of common waterhemp, smooth pigweed (*Amaranthus hybridus* L.), redroot pigweed, slender amaranth (*Amaranthus viridis* L.), and Palmer amaranth was higher at alternating temperatures of 25/20, 30/15, 35/20, 35/30, and 35/25, when compared with constant temperatures of 15, 20, 25, 30, and 35 C (Guo and Al-Khatib 2003; Leon et al. 2004, 2007; Steckel et al. 2004; Thomas et al. 2006). Furthermore, temperature fluctuations decrease with increasing soil depth, thus acting as a depth-sensing mechanism for weed seeds (Baskin and Baskin 1998; Ghersa et al. 1992; Kegode et al. 1998).

Light is required for dormancy alleviation and germination of small-seeded weeds, including *Amaranthus* species that can emerge only from shallow depths (Baskin and Baskin 1977, 1998; Benech-Arnold et al. 2000; Gallagher and Cardina 1998a,b; Leon and Owen 2003). Phytochrome-regulated seed germination of redroot pigweed, smooth pigweed, and common waterhemp has been documented (Leon and Owen 2003; Gallagher and Cardina 1998a,b). Exposure to R light induced germination in dormant seeds of redroot pigweed, smooth pigweed, and common waterhemp, while FR light inhibited germination and induced dormancy (Leon and Owen 2003; Gallagher and Cardina 1998a). The inhibitory effect of FR light was due to a decrease in Pfr/Pr of the

phytochrome, thus causing photodormancy in seeds (Benech-Arnold et al. 2000; Frankland and Taylorson 1983).

Light requirement for germination can vary depending on the dormancy level of seeds, which is further influenced by factors such as burial and temperature (Leon and Owen 2003; Gallagher and Cardina 1998a). Depth-mediated seasonal changes in dormancy and germination have been reported in annual weed species like redroot pigweed and velvetleaf (*Abutilon theophrasti* Medicus) (Benvenuti et al. 2001; Milberg and Andersson 1997). Burial induced a R-light requirement for germination and resulted in a shift from low fluence response (LFR) to very low fluence (VLFR) response of the phytochrome in redroot pigweed and smooth pigweed seeds (Gallagher and Cardina 1998a). The stimulatory effect of R light for common waterhemp germination was also more pronounced in seeds subjected to chilling (moist stratification) at 4 C (Leon and Owen 2003). In addition, high temperatures (30 C or above) during summer can reduce the photosensitivity of *Amaranthus* seeds and overcome the phytochrome mediated R-light requirement for enhanced germination (Gallagher and Cardina 1998a; Hartzler et al. 1999; Leon and Owen 2003).

Knowledge of the germination ecology of weed seeds is essential to implement sound weed management strategies. The effect of temperature and light on seed germination of *Amaranthus* species have been characterized by previous researchers (Baskin and Baskin 1977,1998; Gallagher and Cardina 1998a,b; Leon and Owen 2003; Steckel et al. 2004). However, research on the temporal differences in germination

behavior of these species, especially Palmer amaranth, in response to environmental factors is lacking. Palmer amaranth is one of the most troublesome and competitive weeds of crops in the southeastern United States (Jha et al. 2008a,b; Norsworthy 2003; Webster and MacDonald 2001). The objectives of this research were to determine the role of temperature and light on germination of Palmer amaranth seeds over a 12-month period in field conditions with and without spring burial.

Materials and Methods

Mature seeds of Palmer amaranth were collected from a natural population of plants near Pendleton, SC, in late October of 2004 and 2006. Seeds were allowed to air-dry at 25 C for 7 to 10 d before they were tested for germination or placed in fine mesh nylon bags (20 by 20 cm). In November 2004 and 2006, nylon bags containing approximately 10,000 Palmer amaranth seeds were placed on the soil surface inside polyvinylchloride (PVC) pipes. The pipes were 50 cm in diam and 30 cm in length and buried to a depth of 15 cm in a field at the Clemson University Simpson Research Center in Pendleton, SC. The end of the pipes were covered with metal mesh to prevent any disturbance or predation of seeds by animals. Mean monthly minimum and maximum temperatures during the study period (Figure 3.1) were recorded at a weather station near the experimental site.

One-half of the bags in PVC pipes kept on the soil surface were buried to a 10-cm depth on May 15, 2005 and 2007 to simulate soil incorporation by tillage of seeds that have remained on the soil surface since the previous fall. Spring tillage and planting operations often result in seed burial beneath the soil surface into a much different environment. The remaining seed bags were left on the soil surface in the spring and beyond to simulate no-till conditions in the field. Seeds on the soil surface were evaluated five times over a 12-mo period (0, 3, 6, 9, and 12 mo after maturation), while buried seeds were evaluated only twice (9- and 12-mo after maturation). Retrieval of buried seeds was performed at night under a dim green light obtained by covering a flash

light with a single layer each of blue and yellow filters (Blue filter, Roscolux No. 64; yellow filter, Roscolux No. 10, Rosco, Port Chester, NY 10573).

Following recovery, seeds were wrapped with two layers of aluminum foil and kept inside a dark box to prevent light exposure during transfer from the field to the laboratory. All laboratory manipulations of exhumed seeds were carried out under the previously described green light. Seeds were separated from the soil by sieving and further rinsed with deionized water containing 0.5% (v/v) bleach. Only non-germinated intact seeds were used for the light and temperature experiments. Fifty seeds were placed in a 9-cm-diam petri dish between filter paper, and three replications of each treatment were used for all light and temperature experiments. The germination medium was a 1% (v/v) solution of captan (Captan 4-L fungicide, Drexel Chemical Company, P.O. Box 13327, Memphis, TN 38113-0327) in deionized water (total volume of 3 ml per petri dish).

For temperature experiments, seeds were hydrated with the germination medium and incubated in the dark at constant and fluctuating temperatures. Constant temperature treatments included 10, 15, 20, 25, 30, 35, and 40 C and fluctuating temperature (12/12 h minimum/maximum) treatments included 2.5/17.5, 7.5/22.5, 12.5/27.5, 17.5/32.5, 22.5/37.5, and 27.5/42.5 C. After 14-d incubation, germination was evaluated based on radicle protrusion from the seed. Non-germinated seeds were tested for viability using a crush test (Sawna and Mohler 2002).

For the light experiments, seeds were imbibed in the germination medium for at least 8 h prior to imposing the light treatments. Light treatments included (1) R light for 15 min, (2) FR light for 15 min, (3) R light for 15 min, immediately followed by (fb) FR light for 15 min, (4) FR light for 15 min, immediately fb R light for 15 min, (5) natural light, and (6) dark control. All artificial light treatments were performed inside dark chambers illuminated with a R or FR light (Light filters, Roscolux 27 and Roscolux 19, BMI Supply South, 571 Queensbury Avenue, Queensbury, NY 12804) source (Rajapakse et al. 1993) and the irradiance at the seed level was 2.1 and 12 W m^{-2} for R and FR light sources, respectively. In the natural light treatment, petri dishes containing seeds were exposed to photosynthetic active radiation of 690 mol m^{-2} s^{-1} and R:FR ratio of 1.12 (measurements taken at solar noon) on the bench top in a greenhouse. In the dark control treatment, petri dishes containing seeds were wrapped in two layers of aluminum foil. All petri dishes were wrapped with a transparent film (Transparent film, Fisher Scientific, 3970 Johns Creek Court, Suwanee, GA 30024) to minimize moisture loss and placed in a greenhouse maintained at 24 to 30 C. Seeds in all experiments were kept moist by adding captan solution in deionized water as needed to keep the seeds hydrated. Germination and viability were evaluated after a 14-d incubation. The percentage germination of seeds for both experiments was calculated as the number of germinated seeds divided by the total number of viable seeds (germinated and non-germinated), multiplied by 100.

Light and temperature experiments were analyzed separately using a split-split plot design with burial as the main factor, retrieval date as the subplot factor, and light

treatment or mean temperature regimes and temperature amplitude as the sub-subplot factor. In all experiments, germination percentages were arcsine square-root-transformed to achieve normality of the data. A two-way ANOVA model was used for determining the overall significance of main effects and two-way interactions. If the ANOVA suggested a significant main effect or interaction, Fisher's protected LSD test was used for mean separation. Separate analyses were conducted for the buried seed samples since they were evaluated only in the last two retrieval dates each year. The interaction terms involving burial were assessed only for those two retrieval dates that included seeds from the soil surface and buried. All ANOVA and mean separation calculations were performed using PROC GLM in SAS. All significance tests used an alpha level of 0.05.

Results and Discussion

Germination Response to Temperature During After-Ripening

Germination of Palmer amaranth seeds in response to temperature over a 12-mo period was similar for both 2004 and 2006 seed collections. The temperature requirement for germination varied with the time of year (month) after seed maturation (M × T interaction) (Table 3.1). Germination of freshly matured seeds in November at constant temperatures of 25 to 40 C averaged 72% higher than that at 10 to 20 C (Figure 3.2). At fluctuating temperatures, average germination at 17.5/32.5 (25 C mean) to 27.5/42.5 C (35 C mean) was almost three times that at 2.5/17.5 (10 C mean) to 12.5/27.5 C (20 C mean) (Figure 3.3). Thus, Palmer amaranth seeds immediately after maturation were conditionally dormant and had greater ability to germinate at high temperatures (>20 C). Similarly, freshly matured redroot pigweed seeds in November germinated from 4 to 64% at 15/30 to 20/35 C, whereas little to no germination occurred at 6/15 and 10/20 C (Baskin and Baskin 1977). Other summer annuals including common lambsquarters (*Chenopodium album* L.), common ragweed (*Ambrosia artemisiifolia* L.), Pennsylvania smartweed (*Polygonum pensylvanicum* L.), and pitted morningglory (*Ipomoea lacunosa* L.) have also exhibited similar results (Baskin and Baskin 1977, 1987; Norsworthy and Oliveira 2007; Omani et al. 1999). Germination of Palmer amaranth like most other summer annuals; however, does not occur in the field in November (Baskin and Baskin 1977, 1987; Jha et al. 2007, 2008a; Norsworthy and Oliveira 2007), since temperatures are below those required for germination (Figure 3.1).

Exposure to low temperatures during late fall to winter (November to January) caused dormancy reduction of Palmer amaranth seeds, which resulted in a significant ($p < 0.0001$) increase in germination in February, 3 mo after maturation (Figure 3.2 and 3.3). Seeds retrieved from the soil surface in February averaged 30% higher germination at constant temperatures of 20 to 35 C compared to 10 and 15 C. Germination at 22.5/37.5 (30 C mean) and 17.5/32.5 C averaged 36% and was not significantly different from that at 27.5/42.5 and 12.5/27.5 C, but were an average of 30% higher than the germination at 2.5/17.5 and 7.5/22.5 C. These results suggest a high temperature (>20 C) requirement for increased germination of Palmer amaranth seeds in February following dormancy reduction. However, the mean temperature during February at the test site was 13.3/0 and 12.4/0 C in 2005 and 2007, respectively (Figure 3.1). This partially explains why Palmer amaranth exhibits little to no emergence in the field until late spring (April to May) when temperatures >20 C are attained (Jha et al. 2007). These results are consistent with the previous findings that summer annuals like *Amaranthus* species emerging in late spring to summer require exposure to chilling temperatures during winter for seed dormancy alleviation (Baskin and Baskin 1977, 1987; Bouwmeester and Karssen 1992; Karssen 1982). Once dormancy is reduced or lost, seeds tend to germinate at high temperatures (35/20, 30/20, 30/15, 25/15) (Baskin and Baskin 1977, 1987; Faccini and Vitta 2005).

The overall germination of seeds declined in May (6 mo after maturation) compared to February, but it was higher than that in the previous fall (preceding November). Germination of seeds in May at constant temperatures of 10 and 15 C

averaged 14%, which was similar to those at temperatures >20 C. Germination at mean fluctuating temperatures of 10 and 15 C was also similar to that at 20 to 30 C fluctuating. Thus, seeds gained the ability to germinate equally well at temperatures below the optimum range of 25 to 35 C similar to reports by Guo and Al-Khatib (2003) and Wright et al. (1999). Following exposure to winter temperatures, seeds of redroot pigweed, common ragweed, and common lambsquarters had increased germination only at high temperatures (20/35, 20/30, 15/30, 15/25); however, with additional after-ripening for 1 to 3 months in spring, seeds showed increased germination even at low temperatures (6/15 and 10/20 C) (Baskin and Baskin 1977, 1987). A decline in the minimum temperature or broadening of thermal range for germination following seed dormancy reduction during winter months is well documented in summer annuals (Baskin and Baskin 1977, 1987; Benech-Arnold et al. 1990; Bouwmeester and Karssen 1992).

The temperature amplitude by retrieval date interaction for Palmer amaranth germination was significant (Table 3.1). Fluctuating temperatures improved germination over constant temperatures in seeds that were retrieved at 0, 3, and 6 mo after maturation. However, temperature amplitude did not influence germination of seeds retrieved at 9 and 12 mo after maturation (Figures 3.2 and 3.3). These results are consistent with findings of others in that temperature fluctuations can improve seed germination of summer annuals; however, this requirement of temperature fluctuations can vary over the after-ripening period (Cristaudo et al. 2007; Dillon and Forcella 1985; Nishimoto and McCarty 1997; Norsworthy and Oliveira 2007; Thompson and Grime 1983).

The main effect of burial depth was significant for Palmer amaranth germination (Table 3.1). In August, seeds exhumed from the soil surface had an average germination of 9%, which was higher than the 4% average germination of seeds exhumed from a 10-cm depth. Seeds retrieved after 9 mo from the soil surface or 10-cm depth had similar germination percentages at all constant and fluctuating temperatures. An overall decrease in germination was observed when compared to February and May sampling possibly due to induction of secondary dormancy or thermodormancy in seeds exposed to high temperatures during summer. These results are consistent with the field emergence of Palmer amaranth in South Carolina that declines in early August following peak emergence from May to mid-July (Chapter 2). Induction of secondary dormancy in seeds due to high temperatures occurring in late summer has been previously reported in redroot pigweed, common ragweed, witchgrass (*Panicum capillare* L.), and prostrate knotweed (*Polygonum aviculare* L.) (Baskin and Baskin 1977, 1985a, 1990; Kepczynsky and Bihun 2002).

Similar to August, germination in November (12 mo following seed maturation) was 50% higher for seeds retrieved from the soil surface compared to those from the 10-cm depth. The results suggest that Palmer amaranth seeds buried for 3 to 6 months acquired dormancy. Increase in dormancy of seeds buried at a 5- to 10-cm depth for 3 to 12 months has been reported in other *Amaranthus* species (Baskin and Baskin 1977; Omami et al. 1999). The acquisition of depth-mediated dormancy of weed seeds is known to occur due to lack of light transmittance, decrease in thermal fluctuation,

decrease in oxygen, and increase in carbon dioxide with increasing soil depth (Baskin and Baskin 1985b; Benvenuti et al. 2001; Benvenuti and Macchia 1997,1998; Drew 1990; Holm 1972). Furthermore, depth-imposed dormancy is an important strategy for weed seeds that allows their longevity and perpetuation in the soil seedbank (Burnside et al. 1996, Thompson 1987; Benvenuti et al. 2001).

Averaged over amplitudes, germination of surface and buried seeds was highest from 25 to 40 C. A high temperature requirement for germination during November is not practically significant. Palmer amaranth seeds would not germinate in the field during late fall, since temperatures above 25 C are not likely to occur during that time of the year (Figure 3.1).

Germination Response to Light Exposure During After-Ripening

Palmer amaranth seeds collected in 2004 and 2006 had similar germination response to light treatment ($p = 0.15$). Retrieval date (month) also significantly influenced seed germination (Table 3.2). In addition, the retrieval date by light interaction for germination was significant, indicating that light requirements for germination of seeds changed over time. Freshly matured seeds in November exposed to natural light had 16% germination, which was higher than the 6% germination of those incubated in the dark (Figure 3.4). Exposure to R or FR light did not result in significant differences in germination of recently matured seeds.

Following after-ripening for 3 mo, germination in February averaged 33%, which was higher than that of freshly matured seeds (Figure 3.4). There was no effect associated with light treatment ($p = 0.09$). These results suggest that after-ripening during winter caused dormancy reduction of Palmer amaranth seeds and those seeds were able to germinate irrespective of light environment, provided temperature conditions were suitable for germination (seeds in all light experiments were incubated at 24 to 30 C).

Seeds retrieved in May required natural light or R light for increased germination that averaged 20%. Seeds in dark conditions had seed germination reduced to 9%. A light requirement for germination has been previously reported in other *Amaranthu*s species including common waterhemp, redroot pigweed, and smooth pigweed (Baskin and Baskin 1977; Cristaudo et al. 2007; Gallagher and Cardina 1998a, b; Leon and Owen 2003). These results further explain the occurrence of high field emergence of Palmer amaranth in late spring (May) from seeds lying on the soil surface under no-till conditions or ones brought to the soil surface by spring tillage prior to planting. Similar to the previous retrieval dates, germination in May samples did not differ between R and FR light treatments.

The retrieval date by burial interaction for germination was significant (Table 3.2). Seeds retrieved in August from the soil surface had 55% greater germination than those buried at 10-cm depth. This might be because seeds located on the soil surface were exposed to light and diurnal temperature fluctuations, which have reported effects on seed dormancy alleviation of *Amaranthus* species (Baskin and Baskin 1977, 1985b;

Cristaudo et al. 2007; Guo and Al-Khatib 2003; Leon and Owen 2003; Omami et al. 1999; Steckel et al. 2004). Conversely, germination in November did not differ across burial depths.

The light by burial interaction was not significant (Table 3.2), suggesting that seeds on the soil surface or buried for 3 to 6 mo did not differ in light requirements for germination. In August, germination in natural light or R light and in the absence of light were similar and ranged from 10 to 12% and 12 to 15% for buried and surface seeds, respectively. Based on these data, it is suggested that the high temperatures (30 C or above) occurring in August reduce the R-enriched light requirement for Palmer amaranth germination, which is consistent with findings for other *Amaranthus* species including redroot pigweed, smooth pigweed, and common waterhemp (Gallagher and Cardina 1998a; Hartzler et al. 1999; Leon and Owen 2003). Furthermore, differences in R and FR light response were evident by the August retrieval date. Germination of surface and buried seeds was 13 and 18%, respectively, in response to R light, whereas it was reduced to 5 and 12%, respectively, in response to FR light. The inhibitory effect of FR light on Palmer amaranth seed germination in August supports previous findings that Palmer amaranth seedling emergence beneath a soybean canopy declines with a decrease in R:FR ratio following canopy closure in August (Jha et al. 2007, 2008a).

In November, 12 mo after seed maturation, the overall germination of seeds was only 7% (Figure 3.3), which was the lowest among all retrieval dates, indicating that most seeds were dormant. Seeds lying on the soil surface or buried had a natural light or R

light requirement for germination, which averaged 13%. In contrast, germination declined to 3% when seeds were exposed to FR or R fb FR light or kept in darkness (Figure 3.4). This provides evidence of phytochrome-regulated response of Palmer amaranth seed germination. Phytochrome-mediated responses to R-light promoting germination and FR-light inhibiting germination has also been documented in other *Amaranthus* species (Gallagher and Cardina 1998a; Leon and Owen 2003). Furthermore, germination after treatment with FR fb R light was almost four times that after treatment with R fb FR light, indicating that the phytochrome-mediated response of Palmer amaranth seeds to R and FR light is reversible. Similar results were reported for common waterhemp (Leon and Owen 2003). These results suggest that photodormancy would be a mechanism for successful overwintering and perpetuation of surface-lying Palmer amaranth seeds that are buried in late spring by tillage and planting operations. Additionally, the light requirement for germination would be a depth-sensing mechanism for seeds, implying that the buried seeds will germinate following soil disturbance the following spring (Baskin and Baskin 1977, 1985b, 1998; Benvenuti et al. 2001; Gallagher and Cardina 1998a,b).

In conclusion, cyclical changes in germination of Palmer amaranth seeds occurred during the 12-mo period following seed maturation. Light and temperature requirements of seeds changed over a 12-mo period. Dormancy reduction of seeds occurred in early spring followed by a broadening of the thermal range (10 to 40 C). High temperatures during summer induced secondary dormancy and a decrease in seed germination. By late

autumn, seeds required mean temperatures >25 C for increased germination. Burial induced dormancy and light requirement for seeds. Stimulation of Palmer amaranth seed germination by R light and inhibition by FR light was a phytochrome-mediated response. Further research is needed to understand the physiological/chemical mechanisms such as changes in endogenous hormones (ABA and GA) or other biochemical factors that regulate seasonal changes in germination behavior of seeds in the soil seedbank.

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Table 3.1. Two-way ANOVA for determining the main effects of retrieval date (months), mean temperature, temperature amplitude, and burial depth and the two-way interactions on percent germination of Palmer amaranth seeds during a 12-mo period under field conditions.

Source of variation	df[a]	p value
Burial (B)	1	0.007
Month (M)	4	< 0.0001
Temperature (T)	6	< 0.0001
Amplitude (A)	1	0.8332
B[b] × M	1	0.0011
B × T	6	0.0618
B × A	1	0.0627
M × T	24	< 0.0001
A × T	6	0.0766
A × M	4	0.0078

[a] df, degree of freedom.

[b] Interactions involving burial were studied only for the months (retrieval dates) that included seeds from the soil surface and buried.

Table 3.2. Two-way ANOVA for determining the main effects of retrieval date (months), light, and burial depth and the two-way interactions on percent germination of Palmer amaranth seeds during a 12-mo period under field conditions.

Source of variation	df[a]	p value
Burial (B)	1	0.0006
Month (M)	4	< 0.0001
Light (L)	4	< 0.0001
B[b] × M	1	0.0011
B × L	4	0.2006
M × L	16	0.0006

[a] df, degree of freedom.

[b] Interactions involving burial were studied only for the months (retrieval dates) that included seeds from the soil surface and buried.

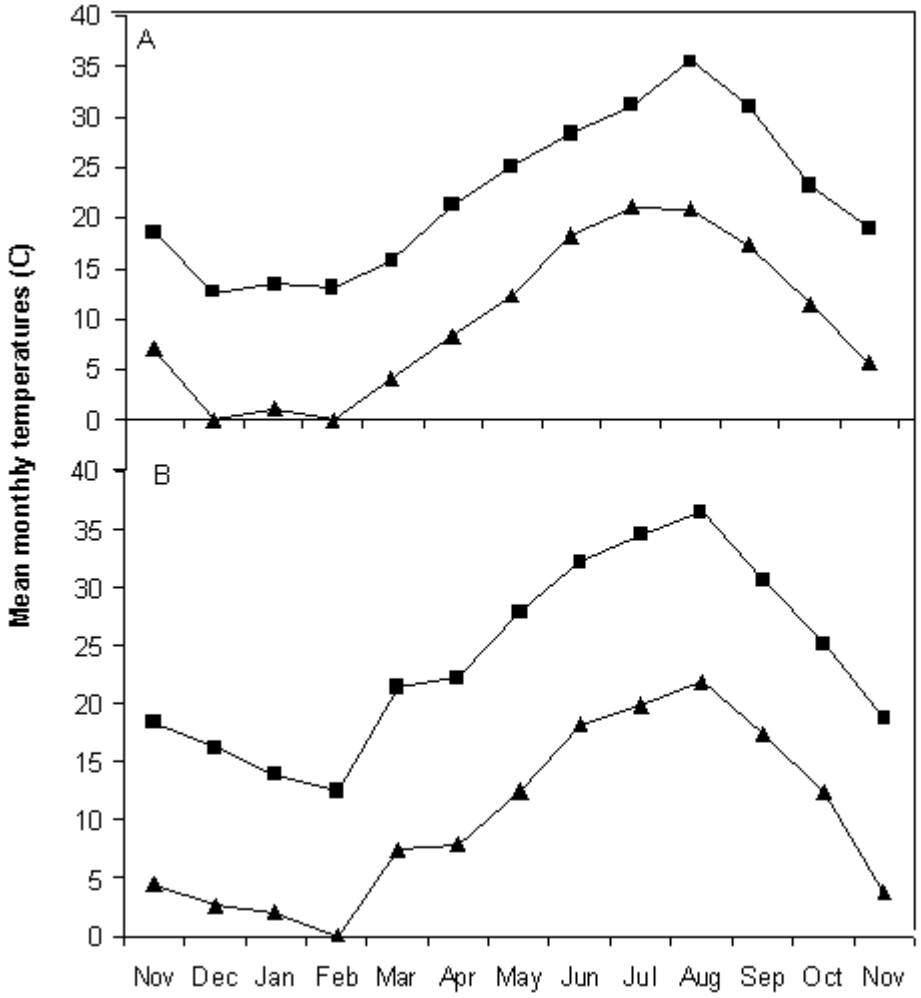

Figure 3.1. Mean monthly maximum and minimum air temperatures from A) November 2004 through November 2005 and B) November 2006 through November 2007 at Pendleton, SC.

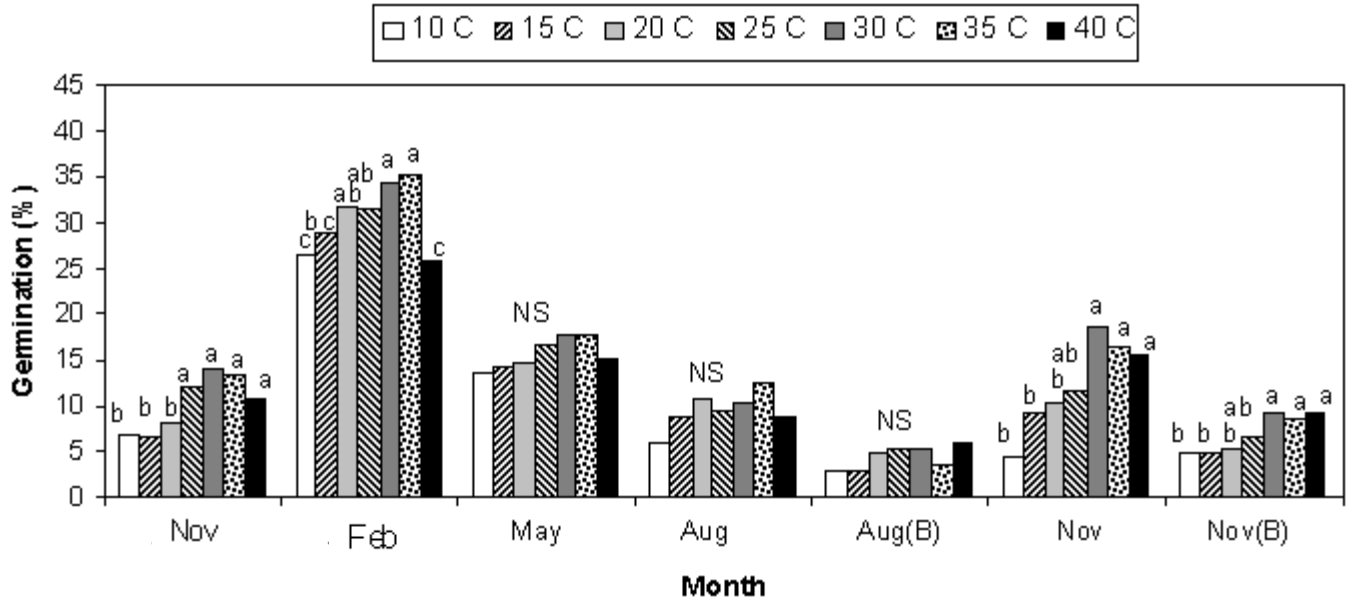

Figure 3.2. Germination percentages of Palmer amaranth in response to constant temperatures over an after-ripening period of 12-mo following maturation in late October averaged across seeds collected in 2004 and 2006. One-half of the nylon bags containing seeds kept on the soil surface were buried to a 10-cm depth in May; therefore, summer and fall evaluations included seeds from the soil surface and from burial (B). Means within each month followed by the same letter are not significantly different based on Fisher's protected LSD at $\alpha = 0.05$.

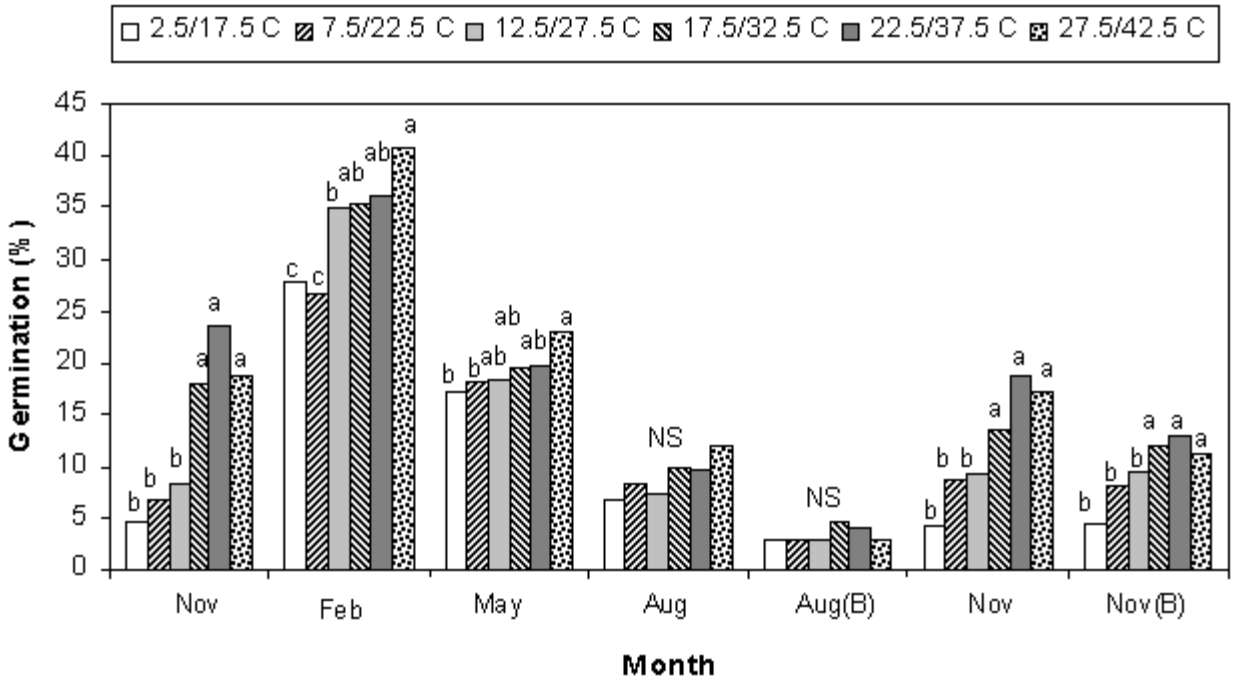

Figure 3.3. Germination percentages of Palmer amaranth in response to fluctuating temperatures over an after-ripening period of 12-mo following maturation in late October averaged across seeds collected in 2004 and 2006. One-half of the nylon bags containing seeds kept on the soil surface were buried to a 10-cm depth in May; therefore, summer and fall evaluations included seeds from the soil surface and from burial (B). Means within each month followed by the same letter are not significantly different based on Fisher's protected LSD at $\alpha = 0.05$.

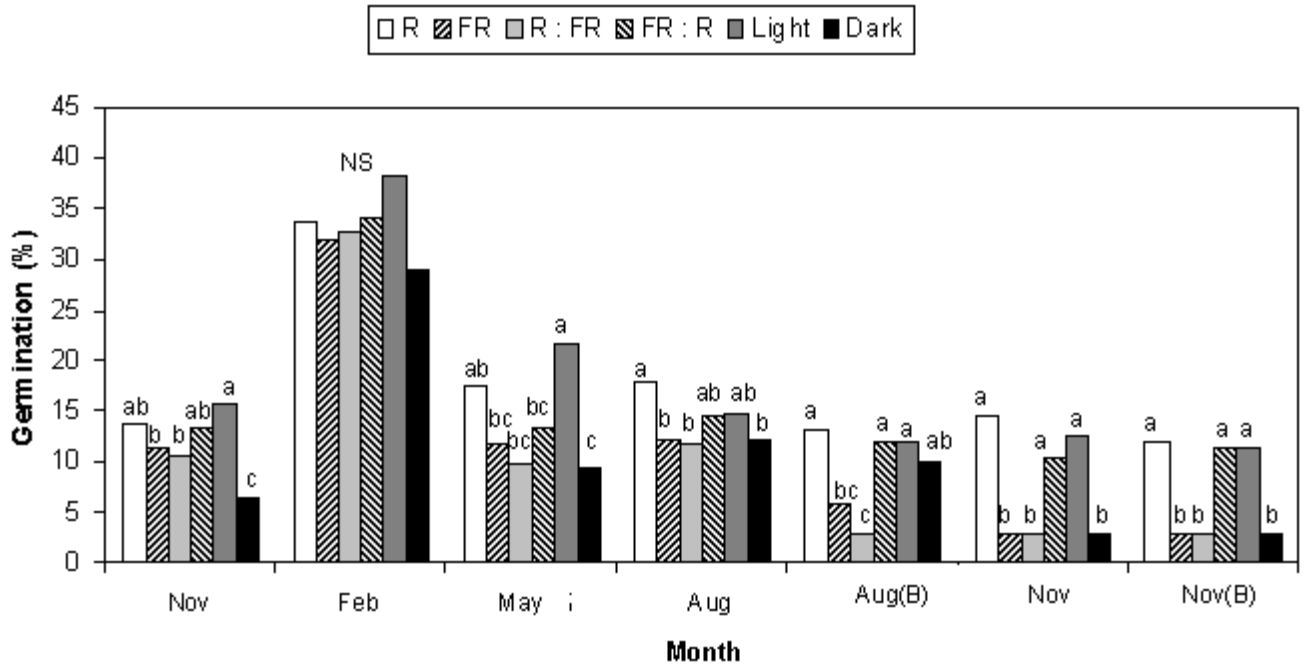

Figure 3.4. Germination percentages of Palmer amaranth in response to light environment over an after-ripening period of 12-mo following maturation in late October averaged across seeds collected in 2004 and 2006. One-half of the nylon bags containing seeds kept on the soil surface were buried to a 10-cm depth in May; therefore, summer and fall evaluations included seeds from the soil surface and from burial. Means within each month followed by the same letter are not significantly different based on Fisher's protected LSD at $\alpha = 0.05$. Abbreviations: B, buried; R, red; FR, far-red light; R:FR, R followed by FR; FR:R, FR followed by R.

CHAPTER 4

SEED DORMANCY OF PALMER AMARANTH AFFECTED BY MATERNAL SHADING AND SEED LOCATION ON THE MOTHER PLANT

Abstract

The effects of maternal shading and seed location on the mother plant on Palmer amaranth seed dormancy were investigated. Increasing the maternal shading from 0 to 87% increased seed dormancy from 75 to 88%. The endogenous gibberillic acid (GA) content of seeds that matured under 47 or 87% shade was 49.7 ng g^{-1} dry seed when compared with 50.5 ng g^{-1} in dry seed that matured under 0% shade (full sunlight). Abscisic acid (ABA) content of progeny seeds increased from 13.3 ng g^{-1} dry seed with 0% shade to 19.1 ng g^{-1} dry seed with 87% shade. Seeds from plants grown under full sunlight or 47% shade had similar endogenous ABA contents. Shading of the maternal plant did not influence the 1000-seed weight of Palmer amaranth. Seed that matured in the bottom third of the mother plant had 91% dormancy compared with 84 to 85% dormancy of those that matured in the top and middle third location of the mother plant. Endogenous GA content of seeds did not differ between locations on the mother plant. However, the ABA content of seeds that were produced in the bottom third of the mother plant was 17.8 ng g^{-1} dry seed, which was higher than the 11.2 and 12.2 ng g^{-1} dry seed in those that were produced at the top and middle portions on the plant, respectively. Seed location on the mother plant had no effect on the 1000-seed weight of Palmer amaranth. Endogenous ABA or GA content of seeds and seed weight had no relationship with seed

dormancy over and above the maternal treatment effects. Thus, it is concluded that Palmer amaranth seed dormancy is partially regulated by maternal shading and seed location on the mother plant.

Introduction

Dormancy is an adaptive trait for weed seeds that enables them to survive conditions unfavorable for germination and seedling establishment (Baskin and Baskin 1998). Seed dormancy influences weed seed bank dynamics and increases the distribution of seedling emergence over time and space (Allen and Meyer 1998; Baskin and Baskin 1998). Weed seed dormancy is often regulated by a compliment of genetic, physiological, and environmental factors (Baskin and Baskin 1998; Karssen 1982, 1986). Factors that occur while seeds are developing on the mother plant are referred to as "maternal effects" (Roach and Wulff 1987).

The environment experienced by the maternal plant during seed maturation and prior to dispersal can result in differences in weed seed dormancy within a population (Baskin and Baskin 1998; Kegode and Pearce 1998; Kigel et al. 1977; Luzuriaga et al. 2006; Munir et al. 2001). Seed dormancy and germination response of wild mustard (*Sinapsis arvensis* L.) varied with nitrogen and water availability to the maternal plants (Luzuriaga et al. 2006). Kegode and Pearce (1998) found that field-raised giant foxtail (*Setaria faberi* Herrm.) and shattercane [*Sorghum bicolor* (L.) Moench.] seeds were less dormant than greenhouse-raised seeds due to greater temperature fluctuations experienced by the maternal plants grown in the field. A difference in photoperiod experienced by the mother plant affected seasonal dormancy of arabidopsis [*Arabidopsis thaliana* (L.) Heynh.] seeds (Munir et al. 2001). Similarly, redroot pigweed (*Amaranthus retroflexus*

L.) seed dormancy was affected by parental photoperiod and temperature environments (Kigel et al. 1977).

Maternal light environment effects on seed dormancy regulation have been documented in some weed species (Bello et al. 1995; Brainard et al. 2005; Kigel et al. 1977). A reduction in maternal light environment [photosynthetic active radiation (PAR)] reduced velvetleaf (*Abutilon theophrasti* Medik.) seed dormancy (Bello et al. 1995). In redroot pigweed, 73% shading of the maternal plant resulted in reduced seed dormancy under short day (8 h light) and increased seed dormancy under long day (16 h light) conditions (Kigel et al. 1977). Brainard et al. (2005) reported a 40 to 50% reduction in dormancy of Powell amaranth (*Amaranthus powelli* S. Watson) seeds maturing under canopy shade (up to 91%) when compared to those maturing under full sunlight.

Seeds maturing in separate locations within an inflorescence or in inflorescences borne at different locations on the mother plant can exhibit variation in their ability to germinate (Baskin and Baskin 1998; Gray and Steckel 1985; Hendrix 1984). A seed-positional effect on dormancy was evident in parsnip (*Pastinaca sativa* L.) seeds originating from primary, secondary, and tertiary umbels (Gray and Steckel 1985; Hendrix 1984). Based on the phenological order of umbel development, seed ripening and maturation in those umbels occurred at different times, exposing the seeds to varying environmental conditions and thus, causing differential dormancy of seeds produced by the plant (Gray and Steckel 1985; Hendrix 1984). El-Keblawy and Al-Ansari (2000)

reported that dormancy and germination responses of common purslane (*Portulaca oleracea* L.) seeds varied due to differences in time of seed maturation on the maternal plant.

Hormonal regulation of weed seed dormancy has been discussed by numerous researchers (Al-Rachedi et al. 2004; Baskin and Baskin 1998; Derkx and Karssen 1994; Hilhorst and Karssen 1992). Dormancy induction occurs during early stages of seed development (Baskin and Baskin 1998; Hilhorst and Karssen 1992). Changes occur in relative amounts of abscisic acid (ABA) and gibberellins (GAs) in the embryo during seed development (Bewly 1977; Hilhorst and Karssen 1992; Karssen and Lacka 1986). ABA concentration increases during initial periods of seed development which then decreases during seed maturity (Baskin and Baskin 1998; Hilhorst and Karssen 1992; Karssen and Lacka 1986). Endogenous levels of ABA and GA in seeds or changes in sensitivity of seeds to these hormones are affected by environmental conditions during seed development (Baskin and Baskin 1998; Walker-Simmons 1987; Romagosa et al. 2001).

Phytohormones (ABA and GA) and environmental factors during after-ripening are known to affect dormancy regulation in *Amaranthus* seeds (Cristaudo et al. 2007; Kepczynski et al. 2003, 2006; Leon et al. 2006, 2007). The direct role of these hormones in dormancy regulation in freshly matured seeds is not clear. Research on the maternal control of seed dormancy in *Amaranthus* species prior to seed dispersal or after-ripening is also lacking. The present research on Palmer amaranth, a member of Amaranthaceae

family, and one of the most troublesome weeds of crops in the southeastern United States (Norsworthy 2003; Webster and MacDonald 2001) will add to our knowledge on weed seed dormancy regulation. It may also help answer the question of why seeds produced from a single plant or a population show differential germination and emergence patterns over time. The objectives of this research were to determine if Palmer amaranth seed dormancy was influenced by seed location on the mother plant, 2) shading of the mother plant, and 3) if endogenous ABA and GA and seed weight play a role in mediating the maternal effects on seed dormancy.

Materials and Methods

Experiments for quantifying the effect of maternal shading and for quantifying the effect of seed location within the maternal plant on Palmer amaranth seed dormancy were conducted at Clemson, SC, in 2006 and 2007. Seeds used for the maternal plants were collected in fall 2005 and 2006 from a natural population of Palmer amaranth at Clemson, SC. Seeds were planted on April 15, 2006 and 2007 in a crop production field on the campus of Clemson University. The soil was a Congaree sandy loam (fine-loamy, mixed active, non-acid, thermic Oxyaquic Udifluvents) with 52% sand, 34% silt, 14% clay, 1.8% organic carbon and a pH of 6.5. For determining the effects of seed location on the maternal plant on dormancy, ABA and GA levels, and seed weight, seeds were planted in a single row and the emerged seedlings were thinned to 10 plants with a spacing of 1-m between plants.

For determining the effects of shading of maternal plants on dormancy, ABA and GA levels, and seed weight, seeds were planted in two rows under 4-m-wide by 8-m-long by 2.5-m-tall shade shelters made from 5-cm-diam polyvinyl chloride frames covered on the top and sides with black shadecloth (Factory Direct Landscape and Greenhouse Supply, 2202 SE 28th PL, Ocala, FL 34471) rated by the manufacturer for 40 and 80% shading. PAR was measured under shade shelters and in the absence of shading at solar noon on a clear day using a line quantum sensor (AccuPar PAR-80 Decagon Devices, Inc., 950 NE Nelson Court, Pullman, WA 99163). The shade cloths rated for 40 and 80% shade intercepted 47 and 87%, respectively, of the incident PAR. Shade levels denoted in

results and tables are based on the actual PAR detected. The shade shelters were open on two opposite ends to allow air circulation. An additional treatment included 0% shading where Palmer amaranth was seeded under direct sunlight. Emerged seedlings were thinned to 6 plants per row with a spacing of 2-m between rows and 1-m between plants. Plants in all experiments were drip-irrigated and fertilized daily with a 0.4% (w/v) nutrient (Scotts Miracle-Gro Products Inc., PO Box 606, Marysville, OH 43040) solution (24% N, 8% P, and 16% K) diluted with water.

At the flowering stage, four Palmer amaranth maternal plants from seed location experiments were selected and the main stem of each of those plants was tagged at three vertically equidistant regions from the base to the apex of the plant. Primary and secondary branch inflorescence arising from each of the three vertical regions on the main stem were tagged and designated as bottom, middle, and top inflorescence. At maturity, tagged inflorescence from each location on the maternal plant were harvested separately. In the shading experiment, inflorescence from four maternal plants under each shade treatment were harvested.

Immediately after harvest in all experiments, inflorescence were air-dried at room temperature, threshed, sieved, and cleaned with an air-column seed cleaner to separate seeds from plant debris. The 1000-seed weight of Palmer amaranth was determined. Germination was evaluated on a portion of the seed lot and the remainder was stored in liquid nitrogen at -80 C for determination of endogenous ABA and GA levels. Seeds

from four replicate plants per treatment were used for the germination tests and analysis of endogenous hormone contents.

Seed germination was evaluated by placing 50 seeds from each treatment in a 9-cm-diam petri dish between filter paper moistened with deionized water containing a 1% (v/v) solution of captan (Tingle and Chandler 2003). Petri dishes were wrapped with a transparent film to minimize moisture loss and incubated in the dark at 30 C. After a 14-d incubation, germination was evaluated based on radicle protrusion from the seed. Viability of non-germinated seeds were determined using a crush test (Sawna and Mohler 2002). Percent (%) dormancy of viable seeds were calculated by using equation 1.

% dormancy= [1- (number of seeds germinated/ total number of viable seeds)] ×100 [1]

For extraction and quantification of endogenous hormones (ABA and GA), 2 g of frozen seeds were spiked with 100 ng each of deuterated (^{2}H)-GA_3 (provided by Dr. Lewis Mander, Research School of Chemistry, Australian National University, Canberra, Australia) and (^{2}H)-ABA internal standards (provided by Dr. L. Irina Zaharia, NRC Plant Biotechnology Institute, National Research Council of Canada, Saskatchewan, Canada) and pulverized under liquid nitrogen. The extraction and purification were done according to the procedure described by Lange et al. (2005) with the modification that the ABA and GAs were first eluted with methanol (6 ml) and then methylated with ethereal diazomethane and dissolved in 200 µl of dichloromethane. The sample was split into equal volumes (50 µl) in two separate vials. One was used for the determination of ABA

content. The second 50 µl volume was dried and trimethylsilated by adding 50 µl of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA). Following evaporation to dryness, the trimethylsilyl derivatized samples were redisssolved in 50 µl of dichloromethane for analysis of GAs. The ABA and GA analyses were performed by GC-MS using a HP5890 gas chromatograph with 5971A mass selective detector (Agilent Technologies, 2850 Ceneterville Road, Wilmington, Delaware 19808). Helium was used as a carrier gas. Samples (1 µl) were injected in the splitless mode at 250 C onto a DB5-MS column (30-m × 0.25-mm i.d.× 0.25-µm) (Agilent Technologies, 2850 Ceneterville Road, Wilmington, Delaware 19808). Injection and interface temperatures were 250 and 300 C, respectively. The oven temperature was kept initially at 100 C for 2 min, increased to 250 C at 20 C min^{-1} and then to 300 C at 4 C min^{-1}. The solvent delay was 5 min and the total run time was 29 min. For quantification of endogenous GA and ABA, single ion monitoring (SIM) for the following characteristics ion pairs (deuterated ion/protio ion) was performed: 506/504 (GA_3), 194/190 (ABA), 166/162 (ABA), 138/134 (ABA). The ratio of peak areas between endogenous [^{1}H]GA_3 and deuterated [^{2}H]GA_3 (m/z 506/504) was used to calculate the amount of endogenous GA. The ratio of peak areas between endogenous [^{1}H]ABA and deuterated [^{2}H]ABA (m/z 166/162) was used to calculate the amount of endogenous ABA. Known GA and ABA standards were also run to confirm retention times of peaks.

All experiments were arranged in a randomized complete block design with years (2006 and 2007) being the blocks and the three maternal shade levels (0, 47, and 87%

shade) or the three seed locations (top third, middle third, and bottom third) on the maternal plant being the treatments. Four maternal plants from each year were used to harvest seeds from each treatment. The individual plants served as subsamples in the randomized complete block design. The analysis was conducted in two steps. The first step was to perform an ANOVA to determine if the treatment (maternal shading or seed location on mother plant) affected the means of dormancy, seed weight, and endogenous ABA or GA contents of Palmer amaranth seeds. If the ANOVA suggested a treatment effect, Fisher's protected LSD test was used to compare treatment means. The second step was to determine if endogenous ABA or GA content of seeds and seed weight had a relationship with seed dormancy over and above the treatment effects. For accomplishing this step, analysis of covariance (ANCOVA) was used with endogenous ABA or GA seed content or seed weight as the covariate. In addition, correlation analysis was conducted within each treatment to further evaluate any possible relationship of dormancy with ABA, GA, or seed weight. Homogeneity of variance and normality assumptions for the data were tested with Levene's test and Shapiro-Wilk's test, respectively. The data met both assumptions. All analyses were performed using PROC GLM of SAS[9] and all significance tests were performed with $\propto = 0.05$.

Results and Discussion

Effect of Maternal Shading.

Palmer amaranth seed dormancy was influenced by the level of shading experienced by the maternal plant ($P = 0.02$). The effect of maternal shading on percent seed dormancy was similar across years. Following incubation in the dark at 30 C for 14 d, seeds from plants grown under 87% shade had 88% dormancy, which was higher than the 75 to 79% dormancy exhibited by seeds from plants grown under full sunlight (0% shade) and 47% shade (Table 4.1). Dormancy did not differ between seeds from plants exposed to 0 and 47% shade. Increased seed dormancy in response to 87% shading of the maternal plant was an adaptive characteristic that would prevent futile or catastrophic germination of Palmer amaranth seeds in a light-limited environment. Furthermore, it would ensure survival and persistence of those seeds in the soil for an extended period of Palmer amaranth seedling emergence in a cropping system (Baskin and Baskin 1998; Bello et al. 1995; Brainard et al. 2005; Jha et al. 2007, 2008; Kigel et al. 1977).

Palmer amaranth seed dormancy in our study was quantified in response to reductions in PAR established through neutral density black shade cloths without altering the red to far-red (R/FR) ratios. In a similar study on redroot pigweed, an increase in seed dormancy in response to 73% shading of the maternal plant grown under long day conditions was reported (Kigel et al. 1977). Decrease in R/FR ratios experienced by plants grown under canopy shade also resulted in increased seed dormancy of *Amaranthus* species such as Powell amaranth (Brainard et al. 2005). Our results further

suggest the possible role of reduced PAR on dormancy of those *Amaranthus* seeds maturing under canopy shade. Contrary to our results and those of others on small-seeded weed species such as *Amaranthus* species (Brainard et al. 2005; Kigel et al. 1977), weed species with large seeds such as velvetleaf exhibited a decrease in seed dormancy in response to increased maternal shading (Bello et al. 1995).

Endogenous GA and ABA concentrations in Palmer amaranth seeds at maturity were influenced by the maternal shade treatment. The ABA and GA contents were averaged across years due to lack of significant difference. Endogenous GA content of the progeny seed was reduced from 50.5 ng g^{-1} dry seed at 0% shade treatment to 49.7 ng g^{-1} dry seed at 47 and 87% shade (Table 4.1). In contrast, ABA content of the progeny seeds increased in response to maternal shading and was significantly different between 0 and 87% shading. The increase however, was not significant between 47 and 0% shade. The endogenous ABA content in seeds that matured under 87% shade was 44% greater than that in seeds that matured in the absence of shade.

Results show that Palmer amaranth maternal light availability can influence the endogenous hormone contents of the seed progeny. Research on the effect of maternal shading or changes in light quantity (PAR) experienced by the mother plant on endogenous hormone concentration in freshly matured seeds is lacking. However, previous researchers have shown that maternal environment can affect endogenous hormone (ABA and GA) concentration in different plant parts including seeds (Benech-Arnold et al. 1991; Wang et al. 2008; Walker-Simmons and Sesing 1990). Wang et al.

(2008) reported an increase in ABA content and decrease in GA_3 content in maize (*Zea mays* L.) seedlings in response to water stress. Water stress during grain filling in shattercane [*Sorghum bicolor* (L.) Moench.] resulted in changes in endogenous ABA and tissue sensitivity to ABA in matured seeds (Benech-Arnold et al. 1991). Likewise, temperature differences experienced by the maternal plant resulted in changes in ABA content in matured wheat (*Triticum aestivum* L.) grains (Walker-Simmons and Sesing 1990).

While the shade treatments affected dormancy, ABA, and GA simultaneously (Table 4.1); the ANCOVA and correlation analyses revealed that there was no additional relationship of dormancy with ABA and GA other than the treatment effect (the p-values for ABA and GA in the ANCOVA and correlation analyses all exceeded 0.30). In other words the variability of dormancy within each treatment could not be easily attributed to variability in ABA and GA. If ABA or GA was a significant cause of the dormancy differences, then we could expect not only for ABA and GA to differ among the treatments, but ABA and GA should vary along with dormancy within the treatments; and this was not the case in this study. Thus, changes in endogenous hormone concentration with shading did not explain the differential dormancy of matured Palmer amaranth seeds. However, differences in tissue sensitivity to hormone could possibly be correlated with differential seed dormancy of Palmer amaranth as evidenced in barley (*Hordeum vulgare* L.) seeds maturing under different environments (Romagosa et al. 2001).

These results are consistent with the findings of previous researchers who reported that there is no clear relationship between endogenous ABA and GA contents and degree of dormancy in freshly matured seeds (Baskin and Baskin 1998; Bradford and Nonogaki 2007; Hilhorst and Karssen 1992; Karssen 1982; Karssen and Lacka 1986; Walker-Simmons 1987). A decrease in ABA concentration in seeds did not show that the seeds were less dormant or that dormancy was broken (Karssen 1982). Previous research has shown that the primary role of ABA during seed development is not to induce dormancy per se but to prevent precocious germination or vivapary of the developing embryo until primary dormancy is induced at seed maturity (Benech-Arnold et al. 1991; Bradford and Nonogaki 2007; Karssen et al. 1983). The results also support previous findings on arabidopsis that GAs are not directly involved in seed dormancy regulation prior to after-ripening (Derkx and Karssen 1994; Karssen and Lacka 1986).

Maternal environment has been shown to influence seed dormancy through changes in seed morphological characteristics, including seed weight (Baskin and Baskin 1998; Gutterman 1978; Luzuriaga et al. 2006; Steadman et al. 2004). In contrast, Palmer amaranth 1000-seed weight was not affected by maternal shading ($P = 0.38$) and was similar for 2006 and 2007. Mean seed weight ranged from 411.3 to 445.6 mg at 0 to 87% shading (Table 4.1). Seed size and seed weight are indicative of soil nutrient and water availability to plants (Luzuriaga et al. 2006). The lack of difference in seed weight due to shading might be because all plants were provided with similar nutrient and water conditions. Moreover, seed weight was not significantly correlated with seed dormancy

in any of the shade treatments [degree of linear relationship (r) ranged from 0.135 to 0.634; p-values > 0.05]. Similarly, in redroot pigweed, seed dormancy affected by the differences in maternal photothermal environment was not correlated with seed weight (Kigel et al. 1977). The lack of a seed weight effect on percent germination of freshly matured seeds has also been reported in other species (Luzuriaga et al. 2006; Wulff et al. 1999).

Effect of Seed Location on the Mother Plant

Palmer amaranth seed dormancy was influenced by the location of seeds on the mother plant (P = 0.02). Dormancy was similar for both years. Seeds produced by inflorescence in the bottom third of the mother plant exhibited 91% dormancy, which was higher than the 84 to 85% dormancy exhibited by those produced at the top and middle portion on the plant (Table 4.2). Effect of seed location on the maternal plant on differential dormancy of seeds has been previously reported (Baskin and Baskin 1979; Gray 1979; Hendrix 1984; Thomas et al. 1978, 1979). The greater dormancy exhibited by seeds produced from the bottom third of the plant was consistent with the increased dormancy exhibited by those produced under 87% shade. This suggests the possible role of reductions in PAR due to mutual leaf shading from the overlying canopy of the plant and in R/FR ratio of leaf canopy transmitted light on increased dormancy of seeds developing at the basal location of the plant. Brainard et al. (2005) and Washitani (1985) also reported a leaf canopy effect on increased seed dormancy of *Amaranthus patulus*

Bertol. and Powell amaranth. These results show that Palmer amaranth seeds dispersed from a single mother plant will likely to exhibit differences in the extent and time of germination in a natural environment.

Flower initiation to complete maturity of Palmer amaranth plants in the field occurred from the first week of August through first weak of November in both the years (2006 and 2007). Not all flowering at the three locations within a mother plant were synchronous and not all seeds produced at those locations matured at the same time or at the same rate, which was expected. The differences in the time of seed maturation at the top and middle compared to the bottom location of the plant might contribute to the variation in seed dormancy. Influence of physiological age or time of seed maturation on the maternal plant on seed dormancy has been previously documented (Baskin and Baskin 1998; Hendrix 1984; Okusanya and Ungar 1983; Thompson 1937). In redroot pigweed, the age of the plant at which flowering was induced resulted in differences in germination response of the progeny seeds (Kigel et al. 1979). El-Keblawy and Al-Ansari (2000) found that the percent germination of common purslane seeds maturing in November was 30% higher than those maturing in August.

Monthly rainfall at the experimental site during August through November varied from 6.6 to 12.9 cm and 3.2 to 5.7 cm in 2006 and 2007, respectively. The average daily maximum and minimum air temperatures during those months in 2006 and 2007 varied from 33.5/20.9 to 18.3/4.3 C and 36.1/21.8 to 18.8/3.7 C, respectively (NCDC-NESDIS 2008). Those slight to moderate variations in environmental conditions that occurred

when Palmer amaranth seeds were developing across the three locations on the plant might have induced possible effects of time of seed maturation on seed dormancy. Influence of maternal environment (temperature, light, rainfall) during seed development and maturation as a preconditioning factor controlling seed dormancy has been well documented (Baskin and Baskin 1998; Drew and Brocklehurst 1990; Gutterman 1978; Kegode and Pearce 1998; Luzuriaga et al. 2006; Steadman et al. 2004; Weiner et al. 1997). For example, redroot pigweed dormancy was affected by 5 C differences in maturation temperature. Germination at 30 C in the dark was higher when seeds matured at 22/17 compared to 27/22 C (Kigel et al. 1977). The temperature differences during seed maturation in our study were greater than that reported by Kigel et al. (1977), which further suggests the possible effect of temperature during seed maturation on differences in Palmer amaranth seed dormancy within a mother plant.

Palmer amaranth 1000-seed weight was similar across locations on the maternal plant ($P = 0.56$) and ranged from 440.3 to 450.9 mg (Table 4.2). Differences in dormancy could not be attributed to seed weight ($P > 0.3$). Furthermore, no significant correlation of seed weight with dormancy was found within each location on the mother plant. Results support the previous findings on *Amaranthus* and other species that seed weight does not explain variation in dormancy and that it is the least plastic plant reproductive character (Harper et al. 1970; Weiner et al. 1997; Kigel et al. 1977; Luzuriaga et al. 2006; Wulff et al. 1999).

The endogenous ABA and GA content of Palmer amaranth seeds were similar for both years. The GA content of seeds was not influenced by the location of seeds on the mother plant ($P = 0.28$) and ranged from 49.9 to 50.3 ng g^{-1} dry seed (Table 4.2). However, the effect was significant on the endogenous ABA content of seeds ($P < 0.0001$). The ABA contents of seeds produced at the top and middle third locations on the plant were similar. Seeds at those locations had an average of 34% less ABA than those in the bottom third of the plant.

While the seed location on the maternal plant affected dormancy, ABA, and GA simultaneously (Table 4.2); the ANCOVA and correlation analyses revealed that there was no additional relationship of seed dormancy with ABA and GA other than the treatment effect (the p-values for ABA and GA in the ANCOVA and correlation analyses all exceeded 0.40). In other words the variability of dormancy within each location on the mother plant could not be easily attributed to variability in ABA and GA. This shows that the higher dormancy of Palmer amaranth seeds located at the bottom third of the plant was not because those seeds had higher ABA concentration. These results agree with the findings of previous researchers that endogenous concentrations of ABA and GA do not necessarily explain differential dormancy of seeds (Baskin and Baskin 1998; Benech Arnold et al. 1991; Bradford and Nonogaki 2007; Hillhorst and Karssen 1992; Karssen 1982).

Based on these results, it can be concluded that maternal shading up to 87% increased Palmer amaranth seed dormancy. Seeds that matured at the bottom third on the

plant were more dormant when compared to seeds from the top and middle third locations. Endogenous hormone (ABA and GA) contents of freshly matured seeds were affected by the maternal light environment and seed location within the mother plant. However, hormone concentrations did not fully explain differences in seed dormancy. Furthermore, no correlation between seed weight and seed dormancy was found.

This research on the maternal effects on Palmer amaranth seed dormancy adds to knowledge on ecophysiological mechanisms for differential seed dormancy within a population that influences the extent and period of weed seedling emergence. Future research is needed to investigate the effects of changes in maternal environment such as light quality, photoperiod, temperature, and nutrient availability on Palmer amaranth seed dormancy. Research is also needed to determine if differences in seed coat thickness, structure, and permeability as functions of the maternal environment could explain for variations in Palmer amaranth seed dormancy prior to after-ripening.

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Table 4.1. Effect of maternal shading on percent dormancy, endogenous hormone contents, and seed weight of Palmer amaranth seeds and correlation between seed dormancy, hormones, and seed weight in each treatment averaged across years (2006 and 2007) at Clemson, SC [b].

	Means [b]				Correlation					
Shading	Dormancy	GA	ABA	1000-seed wt	Dormancy, GA		Dormancy, ABA		Dormancy, seed wt	
%	%	ng g^{-1}	ng g^{-1}	mg	r	p-value	r	p-value	r	p-value
0	74.8 b	50.5 a	13.3 b	445.6 a	0.037	(0.936)	0.263	(0.569)	0.135	(0.772)
47	78.8 b	49.7 b	16.7 ab	449.3 a	0.452	(0.308)	0.444	(0.318)	0.243	(0.600)
87	87.7 a	49.7 b	19.1 a	411.3 a	0.621	(0.266)	0.446	(0.375)	0.634	(0.177)

[a] Abbreviations: ABA, abscisic acid; GA, gibberellic acid; wt, weight.

[b] Means within a column followed by same letter are not significantly different based on Fisher's protected LSD at $\propto = 0.05$.

Table 4.2. Effect of seed location on the maternal plant related to percent dormancy, endogenous hormone contents, and seed weight of Palmer amaranth seeds and correlation between seed dormancy, hormones, and seed weight in each treatment averaged across years (2006 and 2007) at Clemson, SC [a].

	Means [b]				Correlation					
Location on mother plant	Dormancy	GA	ABA	1000-seed wt	Dormancy, GA		Dormancy, ABA		Dormancy, seed wt	
	%	ng g^{-1}	ng g^{-1}	mg	r	p-value	r	p-value	r	p-value
Bottom third	90.6 a	50.3 a	17.8 a	440.3 a	0.687	(0.088)	0.014	(0.978)	0.583	(0.225)
Middle third	84.6 b	50.2 a	12.2 b	450.9 a	0.064	(0.891)	0.216	(0.642)	0.579	(0.228)
Top third	83.7 b	49.9 a	11.2 b	447.8 a	0.055	(0.906)	0.154	(0.742)	0.063	(0.906)

[a] Abbreviations: ABA, abscisic acid; GA, gibberellic acid; wt, weight.

[b] Means within a column followed by same letter are not significantly different based on Fisher's protected LSD at $\propto = 0.05$.

CHAPTER 5

ACCLIMATION OF PALMER AMARANTH TO SHADING

Abstract

Experiments were conducted to investigate the acclimation of Palmer amaranth to shading. Plants were grown in the field beneath black shade cloths providing 47 and 87% shade and in full sunlight (no shading). All photosynthetic measurements were taken 4 wks after initiating the shade treatments. Photosynthetic rates of Palmer amaranth grown under 47% shade increased with increasing photosynthetic active radiation (PAR) similar to 0% shade grown plants. Light-saturated photosynthetic rates were predicted beyond the highest measured PAR of 1200 μmol m^{-2} s^{-1} for plants grown under 0 and 47% shade. Plants acclimated to increased shading by decreasing light-saturated photosynthetic rates from 60.5 μmol m^{-2} s^{-1} under full sun conditions to 26.4 μmol m^{-2} s^{-1} under 87% shade. Plants grown under 87% shade lowered their light compensation point. Rate of increase in plant height was similar among shade treatments. Plants responded to increased shading by a 13 to 44% reduction in leaf appearance rate (leaf number GDD^{-1}) and a 22 to 63% reduction in main stem branch appearance rate (main stem branch number GDD^{-1}) compared with full sunlight. Palmer amaranth specific leaf area increased from 68 to 97 cm^2 g^{-1} as shading increased to 87%. Plants acclimated to 47% shade by increasing total leaf chlorophyll from 22.8 μg cm^{-2} in full sunlight to 31.7 μg cm^{-2} when shaded; however, the increase was not significant at 87% shading. Thus, it is concluded that Palmer amaranth shows photosynthetic and morphological acclimation to 87% or less shading.

Introduction

Palmer amaranth, a dioecious summer annual, is one of the most problematic weeds in row crop production in the southeastern United States (Norsworthy 2003; Webster and MacDonald 2001). It is known to cause severe interference and yield reductions in cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), and soybean [*Glycine max* (L.) Merr.] (Bensch et al. 2003; Keeley and Thullen 1989; Klingaman and Oliver 1994; Massinga et al. 2003). The success of this weed is attributed to its extended period of emergence, prolific growth at high light intensities and high temperatures, and seed production up to 600,000 seeds per female plant (Guo and Al-Khatib 2003; Horak and Loughin 2000; Jha et al. 2007; Keeley et al. 1987; Massinga et al. 2003; Sellers et al. 2003; Weaver 1984). The severity of the problem is further exacerbated due to evolution of herbicide-resistant Palmer amaranth biotypes, with glyphosate-resistant biotypes being the most recent (Culpepper et al. 2006; Gossett et al. 1992; Heap 1997; Horak and Peterson 1995; Mueller et al. 2006; Norsworthy et al. 2008; York et al. 2007).

Light plays a significant role in plant growth and is crucial to crop-weed competition. Shading causes a decrease in the amount of available photosynthetic active radiation (PAR) and adversely affects the growth of plants beneath the canopy (McLachlan et al. 1993a, 1993b; Steckel et al. 2003; Stoller and Myers 1989). Weeds show physiological and morphological adaptations to reduced irradiance and the "adaptive plasticity" to adjust to a light-limited environment (Stoller and Myers 1989). *Amaranthus* species like Palmer amaranth have a C_4 photosynthetic pathway (Ehleringer

1983; Pearcy and Ehleringer 1984; Stoller and Myers 1989). Previous research show that C_4 plants have higher light-saturated photosynthetic rates and are better adapted to high levels of irradiance compared with C_3 plants that saturate at relatively lower light intensities (Regnier and Harrison 1993; Stoller and Myers 1989).

At moderate (< 50%) levels of shading, C_3 species like velvetleaf (*Abutilon theophrasti* Medik.) maintained growth similar to that in full sunlight, whereas, in C_4 species such as common waterhemp (*Amaranthus rudis* Sauer), growth and biomass were reduced even at 40% shade (Bello et al. 1995; Steckel et al. 2003). *Amaranthus* species including common waterhemp, redroot pigweed (*Amaranthus retroflexus* L.), and Powell amaranth (*Amaranthus powellii* S. Wats.) responded to shading by decreasing biomass and leaf appearance rates and by increasing specific leaf area and stem elongation (Brainard et al. 2005; McLachlan et al. 1993a; Steckel et al. 2003). Increased partitioning of biomass to main stem rather than to branch components was reported in redroot pigweed in response to corn-induced shading (McLachlan et al. 1993b).

Weed species with a C_3 photosynthetic pathway such as velvetleaf, common lambsquarters (*Chenopodium album* L.), and eastern black nightshade (*Solanum ptychanthum* Dunal) acclimated to shading by lowering their net photosynthetic rates, light compensation points, and dark respiration rates (Colquhoun et al. 2001; Stoller and Myers 1989). Decreased light compensation points and respiration rates in response to shade have also been reported in some C_4 species such as tumble pigweed (*Amaranthus albus* L.), yellow nutsedge (*Cyperus esculentus* L.), and purple nutsedge (*Cyperus*

rotundus L.), which allow them to maintain a positive carbon balance under prolonged shading (Santos et al. 1997; Stoller and Myers 1989). Shade-adapted leaves respond to low light environments by increasing the total chlorophyll content and decreasing the chlorophyll a:b ratio (Dias-Filho 2002).

Characterizing the physiological and morphological response of weeds to shading would provide valuable information for improving our mechanistic models of crop-weed competition and weed population dynamics (Brainard et al. 2005). Understanding shade response of weeds may also aid selection of crop planting densities and row spacings for reducing weed interference, especially from those cohorts that emerge after crop emergence. Based on the previous research on C_4 species, it was expected that Palmer amaranth would be shade sensitive; however, documented research on shade acclimation of Palmer amaranth is lacking. The objectives of this research were to investigate the effects of shading on Palmer amaranth photosynthetic light response and growth characteristics that are typically associated with shade acclimation.

Materials and Methods

Palmer amaranth seeds collected at Clemson, SC, in fall 2006 were planted in June and July 2007 into 6-L plastic pots containing commercial peat moss mix (Middle weight Mix #3-B, Fafard, Inc., 1471 Amity Road, Anderson, SC 29621) in a greenhouse maintained at 32/24 C day/night temperature with a 16-h daylength. Emerged seedlings were thinned to one plant per pot and were hand-irrigated twice daily. At the four-leaf stage of Palmer amaranth, pots were transferred to the field and plants were grown under 3-m-wide by 2.5-m-long by 2.5-m-tall shade shelters made from 5-cm-diam polyvinyl chloride frames covered on the top and sides with black shadecloth (Factory Direct Landscape and Greenhouse Supply, 2202 SE 28th PL, Ocala, FL 34471) rated by the manufacturer for 40 and 80% shading. The shade shelters were open on two ends to allow air circulation. An additional treatment included 0% shading where plants were grown in full sunlight. Photosynthetic active radiation (PAR) was measured under each shade level as well as in the absence of shading at solar noon on a clear day using a line quantum sensor (AccuPar PAR-80 Decagon Devices, Inc., 950 NE Nelson Court, Pullman, WA 99163). The shade cloth rated at 40 and 80% shade intercepted 47 and 87%, respectively, of the incident PAR. Shade levels denoted in results and tables are based on the actual PAR detected. Plants were watered daily and fertilized with a 0.4% (w/v) nutrient (Scotts Miracle-Gro Products Inc., PO Box 606, Marysville, OH 43040) solution (24% N, 8% P, and 16% K) diluted with water.

Daily minimum and maximum temperatures were recorded at a weather station approximately 1.5 km from the experimental site. Growing degree days (GDD) were calculated as the mean of daily minimum and maximum air temperature minus a base temperature of 10 C for Palmer amaranth (Horak and Loughin 2000). Plant height, number of leaves, and main stem branches were recorded once weekly for 4 wk after subjecting the plants to various levels of shading. After 4 wk, photosynthetic measurements were taken on the two most recently fully expanded leaves on each plant within each shade treatment using a portable photosynthetic system (PP Systems International, Ltd., 110 Haverhill Road, Suite 301, Amesbury, MA 01913). Photosynthetic rates at each shade treatment were determined at 30 C, 19 mbar moisture, 380 ppm external CO_2 concentration (ambient), and at PAR levels of 0 (dark respiration), 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 800, 1000, and 1200 μmol m^{-2} s^{-1}. Immediately following photosynthetic measurements, one of the two sampled leaves were excised from each plant and the leaf outline was traced on paper to determine leaf area using the area to mass relationship of the paper. After drying at 60 C for 48 h, leaf dry weight was measured and specific leaf area (SLA) was calculated as the ratio of leaf area to leaf weight expressed in cm^2 g^{-1}.

The second sampled leaf used for photosynthetic measurement was excised from each plant for chlorophyll determination. Seven 1 cm^2 discs punched from the leaf were homogenized (Brinkmann Instruments, One Cantiague Road, PO Box. 1019, Westburry, NY 11590) at 4 C in 3 ml 95% ethanol with a 10 mm saw tooth probe. Samples were

wrapped in aluminum foil to prevent photo-degradation of chlorophyll. After 15 min of extraction, samples were centrifuged at 12,000 × g for 10 min in a refrigerated centrifuge (Thermo Fisher Scientific Inc., 81 Wyman Street, Waltham, MA 02454) and the supernatant was transferred to a separate tube. Using 95% ethanol as a blank, the absorbance of the chlorophyll extract was recorded on a UV/VIS spectrophotometer (Jasco International Co., Ltd. 4-21, Sennin-Cho 2-Chome, Hachioji, Tokyo 193-0835, Japan) at 665 and 649 nm wavelengths (Winterman and De Mots 1965). Samples were diluted with 95% ethanol when necessary to obtain an absorbance value less than 1.5. The amount of chlorophyll a and b were estimated using Equations 1 and 2, respectively,

$$\text{Chlorophyll a} = 13.70 \times A_{665} - 5.76 \times A_{649}\ \mu g\ ml^{-1} \qquad [1]$$

$$\text{Chlorophyll b} = 25.80 \times A_{649} - 7.60 \times A_{665}\ \mu g\ ml^{-1} \qquad [2]$$

where the coefficients were empirically determined for ethanol (Winterman and De Mots 1965). A_{665} and A_{649} were the absorbance values at wavelengths 665 and 649 nm, respectively.

Chlorophyll a and b concentrations were determined by multiplying the respective value obtained from Equation 1 and 2 by the sample dilution factor and total volume (3 ml). The amount of chlorophyll a or b per unit leaf area ($\mu g\ cm^{-2}$) was calculated by dividing the chlorophyll a or b concentration by the leaf area (7 cm^2) extracted. The amounts of chlorophyll a and b obtained were added to give the total chlorophyll content per unit leaf area.

The experiment was arranged in a randomized complete block design with three shade treatments (0, 47, and 87% shade) and the two planting dates (June and July) being the blocks in the randomized complete block design. Four plants from each planting date were subjected to each shade treatment. The individual plants served as subsamples in the randomized complete block design. Within each shade treatment and planting date, nonlinear regression analyses were performed on photosynthetic rates for each plant as a function of PAR using the nonlinear equation: $y = (a - b) \times \exp(-c \times PAR)$, where y is the predicted photosynthetic rate; a is the predicted light saturated photosynthetic rate; $a - b$ is the leaf-level dark respiration; and $-\log(a / b) / c$ is the light compensation point. This analysis was accomplished using PROC NLMIXED of SAS. Also within each shade treatment and planting date, for each plant, simple linear regression analyses were performed on plant height, leaf number, and main stem branch number as a function of GDD. This analysis was accomplished using PROC REG of SAS. Estimates of the nonlinear and simple linear model parameters were used as predicted photosynthetic parameters (light saturated photosynthetic rate, dark respiration rate, light compensation point) and growth rate parameters (rate of increase in plant height, rate of leaf and main stem branch appearance) for each plant. Analyses of variance (ANOVA) was performed to determine the effect of shading and planting date on those photosynthetic and growth rate parameters and other physiological parameters such as specific leaf area and leaf chlorophyll content. The calculations for this analysis were accomplished using PROC MIXED of SAS based on a randomized complete block design with subsampling model.

Planting dates and plants were considered random effects. Planting date was not a significant term in the model, and therefore, overall means for each shade treatment were reported. Homogeneity of variance and normality assumptions were tested for the estimates with Levene's test and Shapiro-Wilk's test, respectively. The data met both assumptions. Photosynthetic, growth rate, and other physiological parameter means for shading and planting date levels were separated by Fisher's protected LSD test at $\propto =$ 0.05.

Results and Discussion

Photosynthetic Response

Light response curves of Palmer amaranth photosynthesis per unit leaf area showed that plants grown under 47% shade increased photosynthetic rates with increasing PAR similar to those grown under full sunlight (0% shade) (Figure 5.1). Leaves of plants grown under full sunlight or under 47% shade showed no evidence of light-saturation at the highest measured PAR of 1200 μmol m^{-2} s^{-1} provided by the portable photosynthetic unit (Figure 5.1), and hence, the photosynthetic rates were predicted beyond 1200 μmol m^{-2} s^{-1}. Similarly, field-grown redroot pigweed leaves showed no evidence of light-saturation at 2000 μmol m^{-2} s^{-1} and photosynthetic light-saturation intensities greater than 2000 μmol m^{-2} s^{-1} have been reported in other C_4 plants such as corn and grain sorghum [*Sorghum bicolor* (L.) Moench] (Lindquist 2001; Muchow and Sinclair 1994; Sage and Seemann 1993).

The predicted photosynthetic parameters were averaged due to lack of significant differences between planting dates. Predicted light-saturated photosynthetic rate of Palmer amaranth grown without shade was 60.5 μmol m^{-2} s^{-1} (Table 5.1), which was consistent with the rates ranging from 51 to 64 μmol m^{-2} s^{-1} reported for corn and grain sorghum (Lindquist 2001; Louwerse et al. 1990; Muchow and Sinclair 1994; Sinclair and Horie 1989). High light-saturated photosynthetic rates allows Palmer amaranth to be well adapted to high light intensity environments (Pearcy and Ehleringer 1984).

Predicted light-saturated photosynthetic rates were reduced to 11 and 56% in plants grown under 47 and 87% shade, respectively (Table 5.1). Under saturating levels of light, photosynthetic rate of corn grown under 77 and 80% shade was reduced to 28 and 37%, respectively, compared with those grown under full sunlight (Usuda et al. 1985). A reduction in light-saturated photosynthesis in response to shading has also been reported in other C_4 plants such as *Brachiaria* spp., redroot pigweed, sorghum, and tumble pigweed (Dias-Filho 2002; Singh et al. 1974; Stoller and Myers 1989).

The predicted leaf dark respiration rate of Palmer amaranth in full sunlight was 2.95 μmol m^{-2} s^{-1}. When plants were grown under 47% shade, leaf dark respiration was increased by 39% compared to those in full sunlight. The light compensation point of plants under 47% shade was similar to those under full sunlight. However, plants acclimated to 87% shade by lowering their light compensation point to 27.4 μmol m^{-2} s^{-1}, which was 24% lower than that in full sunlight (Table 5.1). Similarly, corn plants grown under 77 and 80% shade had lower light compensation points than those grown under direct sunlight (Usuda et al. 1985). A low light compensation point and/or low dark respiration rate are well known mechanisms of shade acclimation that help plants conserve photosynthates and maintain a positive carbon balance (Colquhoun et al. 2001; Dias-Filho 2002; Stoller and Myers 1989).

Specific Leaf Area

Palmer amaranth showed morphological response to shading by increasing its specific leaf area. Although the increase was not significant at 47% shade compared with full sunlight, plants in 87% shade had a specific leaf area of 97 cm^2 g^{-1}, which was 28 and 42% greater than that in 47 and 0% shade, respectively (Table 5.2). Thus, plants compensated for reduced photosynthetic rates in shade by increasing the leaf surface area per unit of leaf biomass (specific leaf area) which would allow greater harvest of the available PAR per leaf. These results are consistent with the findings of Brainard et al. (2005) who reported that Powell amaranth acclimated to canopy induced reductions in PAR by increasing specific leaf area. Similar leaf adaptations to reduced irradiance have been reported in other *Amaranthus* species such as common waterhemp, redroot and tumble pigweed, and also in C_3 weed species such as eastern black nightshade, velvetleaf, common cocklebur (*Xanthium strumarium* L.), and common lambsquarters (Colquhoun et al 2001; McLachlan et al. 1993b; Regnier et al. 1988; Regnier and Harrison 1993; Steckel et al. 2003; Stoller and Myers 1989).

Increase in specific leaf area of Palmer amaranth with shading was concomitant with decrease in light-saturated photosynthetic rates per unit leaf area (Tables 5.1 and 5.2). Specific leaf area is indicative of leaf thickness, and plants grown under shade with higher specific leaf area tend to have thinner leaves (Regnier et al. 1988). Thin leaves under shade avoid mutual chloroplast shading and reach photosynthetic light-saturation at lower light intensities owing to the fewer palisade and mesophyll cells per unit leaf area

(Regnier et al. 1988; Singh et al. 1974). In addition, producing thinner leaves with higher specific leaf area under shade allowed Palmer amaranth to lower its light compensation point and dark respiration rate per unit leaf area. Similar association of light-saturated photosynthetic rate and leaf respiration rate with specific leaf area has been reported in other plant species (Patton and Jones 1989; Winzeler et al. 1989).

Leaf Chlorophyll Content

Averaged over planting dates, total leaf chlorophyll content of Palmer amaranth increased from 22.8 μg cm^{-2} in full sunlight to 31.7 μg cm^{-2} under 47% shade and the increase was attributed to 42 and 36% increase in chlorophyll a and b, respectively (Table 5.2). Increase in leaf chlorophyll is a photosynthetic acclimation of plants to low-light environments (Dias-Filho 2002; Regnier et al. 1988). No increase in the amount of chlorophyll a or b per leaf area occurred in plants under 87% shade relative to those under full sunlight. Usuda et al. (1985) also reported a lack of difference in the amount of chlorophyll per leaf area in corn plants grown under 80 to 92% shade compared with those grown in full sunlight. Despite increased specific leaf area of plants grown under 87% shade, failure to increase leaf chlorophyll per unit leaf area caused reduction in the photosynthetic ability of those plants.

Growth Rate

All the measured growth rate parameters were averaged over planting dates due to lack of significant interaction terms. Palmer amaranth height increased linearly with GDD (base 10 C) in all shade treatments. The increase in plant height per GDD was not affected by the decrease in PAR due to shading ($P = 0.18$) (Table 5.3), which indicates that plants grown in 47 and 87% shade would require the same amount of thermal time or GDD to reach height similar to those grown in full sunlight (0% shade). Similarly, Steckel et al. (2003) reported that decrease in PAR as a result of increased shading from 0 to 68% had no effect on common waterhemp plant height. Lack of differences in plant height between low (20 and 40%) and high (60 and 80%) levels of shading has also been reported in other C_4 species like yellow nutsedge (Santos et al. 1997). Apart from a decrease in PAR, shading causes a decrease in the red:far-red (R:FR) ratio of transmitted light under a crop canopy, resulting in stem elongation (Brainard et al. 2005; McLachlan et al. 1993b). Lack of a height response to shading in the present study and those conducted by Steckel et al. (2003) and Santos et al. (1997) was likely because shading was established artificially using neutral shade cloths, which were not likely to alter R:FR ratio.

Palmer amaranth leaf number increased linearly with GDD in all shade treatments. However, rate of leaf appearance differed among the three shade treatments ($P < 0.0001$). Plants grown in full sunlight produced 0.39 leaves GDD^{-1} compared with 0.34 and 0.22 leaves GDD^{-1} produced by plants grown under 47 and 87% shade,

respectively (Table 5.3). Thus, plants responded to increased shading by reducing leaf appearance rate. Decreasing the rate of leaf appearance or delaying the development of new leaves with increased shading is an acclimation that allows Palmer amaranth to conserve limited assimilates and maintain growth under low photosynthetic rates. Our results are consistent with findings of McLachlan et al. (1993a), who reported that redroot pigweed emerging late in corn responded to canopy shade by reducing the rate of leaf appearance. Similarly, common waterhemp plants acclimated to shade (up to 68%) by lowering the growth rate and increasing the duration of dry matter accumulation (Steckel et al. 2003). Similar shade acclimation has been reported in other weed species (Deen et al. 1998; Pook 1983).

Main stem branch appearance increased linearly with GDD in all shade treatments and the rates of main stem branch appearance differed among shade treatments ($P < 0.0001$). The rate was greatest in full sunlight (0.051 branches GDD^{-1}). Plants responded to increased shading from 47 to 87% by a 52% reduction in branch appearance rate (Table 5.3). Producing fewer branches is a shade acclimation strategy that allows Palmer amaranth to allocate less of the vegetative biomass to branch components that deplete photosynthates. Furthermore, producing fewer branches when shaded ensures sufficient resources to meet the demands of reproductive development. Similarly, tumble pigweed and redroot pigweed responded to reduced PAR by delaying growth and/or reducing the relative distribution of dry matter to stems and branches (McLachlan 1993a,b; Stoller and Myers 1989).

It is concluded that Palmer amaranth, a C_4 plant, is well adapted to elevated PAR environments because of high light-saturated photosynthetic rates that enables the plant to maintain superior growth rates. Plants showed the ability to adjust their photosynthetic response by lowering their light-saturated photosynthetic rates and light compensation point and/or by increasing leaf chlorophyll content when shaded. Palmer amaranth showed morphological acclimation to shading by increasing its specific leaf area and decreasing its leaf appearance and main stem branch appearance rates. Thus, the present study reveals that Palmer amaranth can acclimate to 87% or less shading and is likely to compete with crops in light-limited environments. Future research is needed on the effects of light quality on growth response of Palmer amaranth and also on the effects of shading on fecundity and seed dormancy of Palmer amaranth that would aid developing models for predicting weed fitness.

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Table 5.1. Effect of shading on photosynthetic response of Palmer amaranth 4 wk after inducing the shading treatments averaged over June and July, 2007 at Clemson, SC.[a]

	Predicted photosynthetic parameters[b]		
Shade level	Light saturated photosynthetic rate	Leaf dark respiration rate	Light compensation point
%	μmol m^{-2} s^{-1}		
0	60.5 a	2.95 b	36.0 a
47	53.6 b	4.11 a	42.8 a
87	26.4 c	2.89 b	27.4 b

[a] Means within a column followed by the same letter are not significantly different based on Fisher's protected LSD test at $\alpha = 0.05$.

[b] Predicted photosynthetic parameters were derived from the nonlinear regression equation $y = (a - b) \times \exp(-c \times PAR)$ where y is the predicted photosynthetic rate; a is the predicted light saturated photosynthetic rate; $a - b$ is the leaf-level dark respiration; and $-\log(a / b) / c$ is the light compensation point.

Table 5.2. Effect of shading on Palmer amaranth specific leaf area and leaf chlorophyll content averaged over June and July, 2007 at Clemson, SC.[a]

Shade level	Specific leaf area	Chlorophyll a	Chlorophyll b	Total chlorophyll
%	$cm^2\ g^{-1}$	$\mu g\ cm^{-2}$		
0	68.2 b	12.5 a	10.2 b	22.8 b
47	75.7 b	17.8 a	13.9 a	31.7 a
87	97.0 a	13.4 a	10.4 b	23.8 ab

[a] Means within a column followed by the same letter are not significantly different based on Fisher's protected LSD test at $\alpha = 0.05$.

Table 5.3. Simple linear regression coefficients for the relationship of Palmer amaranth growth relative to growing degree days (GDD) from 1 to 4 wk after inducing the shading treatments averaged over June and July, 2007 at Clemson, SC.

Shade level	Regression coefficients[a]		
	Intercept	Slope (growth rate)[b]	R^2
%		Plant height	
	cm	cm GDD^{-1}	
0	-7.458	0.132 a	0.971
47	-6.688	0.123 a	0.968
87	-8.370	0.115 a	0.935
		Leaf appearance	
	no.	no. GDD^{-1}	
0	-34.331	0.390 a	0.972
47	-30.689	0.340 b	0.960
87	-20.104	0.220 c	0.955
		Main stem branch	
	no.	no. GDD^{-1}	
0	-2.558	0.051 a	0.987
47	-2.335	0.040 b	0.982
87	-1.329	0.019 c	0.974

Table 5.3 (Continued)

[a] Regression coefficient estimates are obtained from regression equation y = a + bx of each growth parameter (y = plant height, leaf appearance or main stem branch) on growing degree days (x = GDD; base 10 C), where a is the intercept and b is the slope.

[b] Slope coefficients within a column followed by the same letter are not significantly different based on Fisher's protected LSD test at $\alpha = 0.05$.

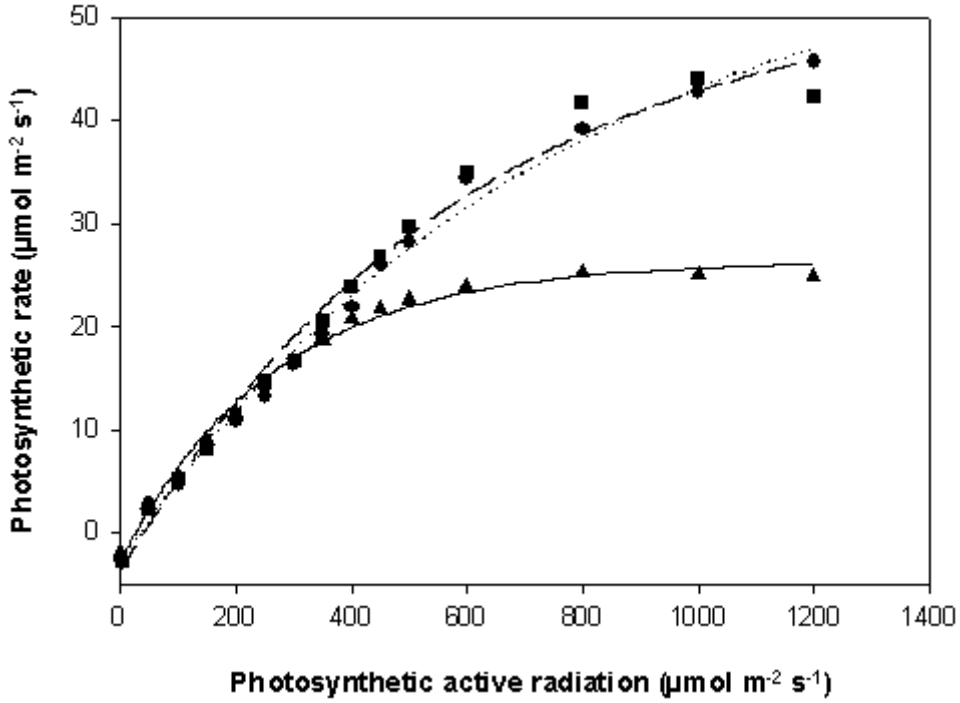

Figure 5.1. Light response curves of Palmer amaranth grown under 0% shade (circles and dotted lines), 47% shade (squares and dashed lines), and 87% shade (upward triangles and solid lines). Fitted lines were calculated from the nonlinear regression model y = (*a* - *b*) × exp(-*c* × PAR), where y is the predicted photosynthetic rate; *a* is the predicted light saturated photosynthetic rate; *a* - *b* is the leaf-level dark respiration; and - log (*a* / *b*) / *c* is the light compensation point. Photosynthetic measurements were taken at 4 wk after inducing the shading treatments.

CHAPTER 6

INFLUENCE OF GLYPHOSATE TIMING AND ROW WIDTH ON PALMER AMARANTH AND PUSLEY DEMOGRAPHICS IN GLYPHOSATE-RESISTANT SOYBEAN

Abstract

The influence of soybean row width and glyphosate application timing was determined on survival, biomass, and seed production of cohorts from a mixed population of Palmer amaranth and pusley species (Florida and Brazil pusley) along with soybean seed yield. The first Palmer amaranth and pusley cohort comprised plants that emerged from soybean planting through the V3 soybean stage (cohort 1). The second cohort comprised plants that emerged between the V3 to V6 soybean stages (cohort 2), and the third cohort emerged after the V6 stage through the R2 soybean stage (cohort 3). Glyphosate at 840 g ae ha^{-1} was applied at V3; V6; V3 and V6; and V3, V6, and R2 in rows either 19 or 97 cm wide. A nontreated control was included for comparison in each row width. Sequential glyphosate applications at V3 and V6; or V3, V6, and R2 soybean stages resulted in 1 to 3% survival of cohort 1 compared with 23 to 28% survival following a single glyphosate application. Vegetative biomass production by cohort 1 accounted for 71% of the total pusley biomass produced in the nontreated plots. Cohort 1, 2, and 3 contributed 68, 31, and 1% of the total 37,900 seeds m^{-2} produced by pusley plants in nontreated plots. Delaying a glyphosate application to the V6 stage resulted in higher biomass and more than twice the seed produced from cohort 1 when compared to cohort 2. Glyphosate applied at V3 and V6 stages prevented pusley seed production from

cohort 1 and an additional glyphosate application at the R2 stage prevented seed production from cohort 2 and 3. No Palmer amaranth emergence occurred after the V6 soybean stage in either row width. A single glyphosate application at the V3 or V6 stage eliminated cohort 1 of Palmer amaranth in narrow rows. Palmer amaranth plants from cohort 1 in wide rows that survived the V3 glyphosate application produced 3.3 g m^{-2} biomass and 600 seeds m^{-2}. Averaged over years and row widths, soybean yields following sequential glyphosate applications were 2,490 to 2,640 kg ha^{-1} compared with 1,850 to 2,020 kg ha^{-1} following a single glyphosate application at the V3 or V6 stage. This research confirms that sequential glyphosate applications are superior to a single application for minimizing pusley and Palmer amaranth survival, biomass, and seed production along with an improvement in soybean yields.

Introduction

Palmer amaranth, a member of the Amaranthaceae (pigweed) family, is a dioecious summer annual and is one of the most troublesome weeds of crops in the southeastern United States (Webster and MacDonald 2001). It is the second most troublesome weed in South Carolina soybean (Norsworthy 2003) and is known to cause severe interference in cotton, corn, and soybean (Keeley and Thullen 1989; Klingaman and Oliver 1994; Massinga et al. 2001). Palmer amaranth at densities of 8 plants m^{-1} row can cause up to 78 and 91% yield reductions in soybean and corn, respectively (Bensch et al. 2003; Massinga et al. 2003). The success of Palmer amaranth as a major weed is attributed to its prolific seed production (up to 600,000 seeds per female plant), extended emergence period that coincides with crop establishment, aggressive growth at higher temperatures due to its extensive root system, thermostability of the C_4 photosynthetic mechanism, and high water use efficiency (Guo and Al-Khatib 2003; Horak and Loughlin 2000; Jha et al. 2007; Keeley et al. 1987; Massinga et al. 2003; Sellers et al. 2003; Weaver 1984). Furthermore, Palmer amaranth resistance to imidazolinone, sulfonylurea, dinitroaniline, and triazine herbicides has been documented (Gossett et al. 1992; Heap 1997; Horak and Peterson 1995). Prior to the recent occurrence of glyphosate resistance in Palmer amaranth (Culpepper et al. 2006; Mueller et al. 2006; Norsworthy et al. 2008; York et al. 2007), glyphosate had been highly effective in controlling this weed (Bond et al. 2006; Norsworthy 2004, 2005; Scott et al. 2002), which contributed to rapid adoption of glyphosate-resistant soybean and cotton throughout the southern United States.

Florida pusley and Brazil pusley are the two major species of pusley ranked among the most troublesome weeds of crops in the southeastern United States (Buchanan 1974; Chandran and Singh 2003; Dowler 1997). Both species belong to the family Rubiaceae and are characterized by the pubescence on stems and leaves. Similar appearance of these two species make them difficult to differentiate at the seedling and vegetative stage. Florida pusley is a summer annual, reproducing sexually by seeds, whereas Brazil pusley is a perennial that can reproduce sexually and asexually (Murphy et al. 1996). Both pusley species are prolific seed producers which germinate well at 20 to 30 C from shallow soil depths (less than 1.5 cm) when the pH ranges from 3 to 8 (Biswas et al. 1975). The severity of Florida and Brazil pusley infestation in glyphosate-resistant crops is attributed to their marginal susceptibility to glyphosate (Chandran and Singh 2003; Reddy and Singh 1992; Sharma and Singh 2001) which is highly dependent on the plant size at time of application (Murdock and Sherrick 1999). Florida pusley is most susceptible to glyphosate when applied to 2.5 to 5 cm plants (Murdock and Sherrick 1999; Reddy and Singh 1992). Brazil pusley control following glyphosate application at 770 g ae ha^{-1} is marginal (14%) and even higher rates do not prevent regrowth of roots and shoots, making it a difficult-to-control weed when using glyphosate in glyphosate-resistant crops (Chandran and Singh 2003; Sharma and Singh 2001).

Economic threshold models based on weed densities often fail to explain crop-weed interference and yield loss based on time of weed emergence relative to the crop (Dieleman et al. 1995; Knezevic et al. 1994; Massinga et al. 2001). At similar densities,

weed cohorts that emerge during early stages of crop development are often more competitive, produce more biomass and seeds, and contribute to greater yield losses than those cohorts that emerge later in the growing season (Dieleman et al. 1996; Knezevic et al. 1994; Massinga et al. 2001; Norsworthy et al. 2007; Steckel and Sprague 2004). The lower competitive ability of late emerging cohorts is mainly attributed to greater interspecific competition and higher mortality of the plants that emerge beneath the canopy and the effect is more pronounced as crop row width is narrowed due to earlier canopy closure and greater light interception (Buehring et al. 2002; Norsworthy et al. 2007). In the absence of residual herbicides, late-emerging cohorts however can contribute significantly to the replenishment of the soil seed bank which warrants the need of sequential glyphosate applications, especially for difficult-to-control weeds in glyphosate-resistant crops (Norsworthy and Oliver 2001; Norsworthy et al. 2007; Payne and Oliver 2000; Steckel and Sprague 2004).

Weed management decisions involving Palmer amaranth and pusley species can be improved with an understanding of the population dynamics and demographic processes such as seedling emergence, survival, biomass, and fecundity of the cohorts emerging at different growth stages of the crop and response of these cohorts to herbicides and other cultural practices (Bussan et al. 2000; Norsworthy et al. 2007; Puricelli et al. 2002). Documented research on the effect of glyphosate on demographics of pusley and Palmer amaranth in wide- and narrow-row glyphosate-resistant soybean is lacking. The objectives of this research were to evaluate the effect of glyphosate timing

and soybean row width on survival, biomass, and seed production of Palmer amaranth and pusley cohorts along with their ultimate impact on soybean seed yield.

Materials and Methods

Field experiments were conducted at the Edisto Research and Education Center near Blackville, SC, in 2004 and 2005 to examine the influence of glyphosate timing and soybean row width on Palmer amaranth and pusley demographics. The soil type during both years was a Dunbar sandy loam (fine, kaolinitic, thermic Aeric Paleaquults) with 0.6% organic matter and pH of 6.0. The test site contained a natural infestation of Palmer amaranth and pusley. The pusley population was a mixture of Florida pusley and Brazil pusley. The experimental area was disk harrowed in mid-May each year and field cultivated prior to soybean planting. Glyphosate-resistant soybean 'Delta & Pineland 7220' was drill-seeded in 18-cm width rows at 432,000 seeds ha^{-1} and in 97-cm width rows at 340,000 seeds ha^{-1} on June 18, 2004 and June 24, 2005. Narrow-row plots were 3 by 9 m, and wide-row plots were 3.9 by 9 m. Immediately following planting, a 1 m^2 quadrat was established in the center of each plot to monitor Palmer amaranth and pusley emergence and survival through soybean harvest. Emerged pusley and Palmer amaranth plants within the quadrat were marked separately using colored tooth picks. Different colored tooth picks were also used to differentiate Palmer amaranth and pusley cohorts that emerged at different soybean growth stages. The first Palmer amaranth and pusley cohort comprised plants that emerged from soybean planting through the V3 soybean stage. The second cohort comprised plants that emerged between the V3 to V6 soybean stages, and the third cohort comprised plants that emerged after the V6 soybean stage through the R2 soybean stage. These cohort designations were reflective of the

glyphosate application timings investigated during this research rather than the occurrence of particular peak emergence periods for any weed.

Glyphosate was applied at 840 g ha^{-1} at V3 [(3 wk after soybean emergence (WAE)]; V6 (5 WAE); V3 and V6; and V3, V6, and R2 (8 WAE) soybean stages (Fehr and Caviness 1977), and a non-treated control for each row width was included for comparison. The test area was kept free from broadleaf weeds other than pusley and Palmer amaranth by hand-weeding, and grasses were controlled by application of sethoxydim at 340 g ai ha^{-1} plus 1% v/v crop oil concentrate at the V5 to V6 soybean stage. Herbicides were applied using a hand-held boom at 5 km h^{-1} calibrated to deliver 94 L ha^{-1} at 276 kPa. The test site was overhead irrigated to encourage weed emergence and minimize moisture limitations to yield. Palmer amaranth and pusley emergence and survival within each cohort was monitored every other week beginning two weeks after planting soybean and continued through soybean harvest.

Photosynthetically active radiation (PAR, mol m^{-2} s^{-1}) was measured within 1.5 h of solar noon at V7 to V8 stage using a line quantum sensor in plots that received three applications of glyphosate. Percent light interception by soybean was calculated using Equation 1,

$$\%LI = (a - b)/a \times 100 \quad [1]$$

where *LI* represents percent light interception by soybean, *a* is PAR measured above the soybean canopy, and *b* is PAR at the soil surface beneath the canopy. The light level at

the soil surface (*b*) was the average of two random readings from each measured plot, taken perpendicular to the planted rows.

Palmer amaranth cohorts matured earlier than pusley and were harvested two to four weeks prior to harvesting soybean to minimize seed loss. Harvest dates of pusley differed among cohorts and the third cohort of pusley was harvested on the day of soybean harvest. Weeds were harvested using hand pruners and the vegetative and reproductive biomass were separated to determine biomass and seed production for each cohort. Harvested Palmer amaranth and pusley vegetative biomass was oven-dried at 60 C for 2 wks and weighed. Harvested Palmer amaranth inflorescence were threshed, sieved two to three times, and air-blown to separate the seeds from bracts. Pusley seeds were separated by crushing the capsules by hand and sieving. Seeds were allowed to air dry on a laboratory bench for approximately 6 mo. The 1000-seed weight of Palmer amaranth and 100-seed weight of pusley was determined to estimate seed production per m^{-2}.

Soybean was harvested from the two center rows in wide-row plots and from the seven center rows in narrow-row plots using a small-plot combine. Percent moisture of soybean seeds was determined, and weights were adjusted to 13% moisture.

The experiments were conducted as a split plot design with soybean row width as the main factor and glyphosate timings as the split plot factor. Each plot contained soybeans along with pusley and Palmer amaranth. Each row width and glyphosate timing treatment combination was replicated in four blocks. Since survival of pusley and Palmer

amaranth within each row width and glyphosate timing treatment combination was monitored in different cohorts and in different WAE, the experimental design also included repeated measures for WAE and cohort. Biomass and seed production of the two weed species in each cohort were measured only at harvest. The experiment was repeated for two years which included another repeated measure factor in the design.

A linear model was developed for the randomized complete block split plot with repeated measures design. It included main effects of year, row width, glyphosate timings, WAE, and cohorts (when applicable), as well as interactions. Error terms were defined for the different splits and repeated measures. ANOVA was used to test the overall significance of the main effect and interaction, and Fisher's protected LSD test was used for mean separation to determine specific differences among factor levels. The assumption of normality was evaluated with the Shapiro-Wilk test and the assumption of equal variances was evaluated with the Levene test. The data met both assumptions. Data were displayed in tables showing main effect (or marginal) means as well as individual combination means when interactions suggested combination means should be studied. All ANOVA and mean separation calculations were performed using SAS PROC MIXED and all assumption test calculations were performed using SAS PROC GLM and SAS PROC UNIVARIATE. All significance tests used an alpha level of 0.05.

Results and Discussion

Pusley emergence in cohort 1, 2, and 3 were similar in both years (2004 and 2005) and soybean row width did not affect emergence in cohort 1, 2, and 3 (P = 0.255, P = 0.165, P = 0.806). Emergence in cohort 1, 2, and 3 averaged 341, 71, and 35 plants m^{-2}, respectively (data not shown). Pusley in cohort 1 ranged from cotyledon to the five-leaf stage and were up to 7 cm tall at the V3 soybean stage in both years. Pusley plants in cohort 1 not treated with glyphosate at the V3 soybean stage were up to 15 cm in height and had up to 12 true leaves by the V6 soybean stage. In both years, pusley in cohort 2 were up to 5 cm tall with five true leaves at the V6 soybean stage. Of the pusley that emerged after planting, 85% of the emergence occurred by the V6 soybean stage.

Palmer amaranth emergence in cohort 1 and 2 were similar across years and were not influenced by soybean row width (P =0.8614, P = 0.6743). Emergence was 100 and 7 plants m^{-2} in cohort 1 and 2, respectively (data not shown). In both years, Palmer amaranth in cohort 1 were up to 35 cm tall with 18 true leaves at the V3 soybean stage. Plants not treated at the V3 soybean stage were up to 80 cm in height and had up to 40 true leaves by the V6 soybean stage. Palmer amaranth that emerged in cohort 2 were up to 25 cm tall with 15 true leaves by the V6 soybean stage. No Palmer amaranth emergence occurred after the V6 soybean stage in either row width, which is consistent with other research where Palmer amaranth emergence from a natural soil seed bank was complete by mid-August in South Carolina (Chapter 2). In a study conducted by Steckel et al. (2001) in central Illinois, common waterhemp (*Amaranthus rudis* Sauer) emergence

began in late April and was complete by mid-July. Similarly, 10 to 95% cumulative emergence of smooth pigweed (*Amaranthus hybridus* L.) in northeastern United States occurred between late April to late July (Myers et al. 2004).

Pusley Survival

Percent survival of pusley that emerged in cohort 1 was influenced by the interaction of glyphosate timing and WAE over which survival was monitored during the growing season ($P < 0.0001$). Survival was similar across years and was not affected by soybean row width ($P = 0.4562$). Two wks following glyphosate application at the V3 soybean stage, survival of cohort 1 was 30 to 41% (Table 6.1). Survival in V6 glyphosate-treated plots was similar to nontreated plots, which was expected because no glyphosate was applied to the V6 plots prior to 5 WAE. Pusley survival at 7 WAE or two wks following a single glyphosate application at the V6 stage was 46%, but further mortality of injured plants resulted in survival similar to the V3 timing by 9 WAE.

Cohort 1 survival 9 WAE following glyphosate applied at V3 and V6; or V3, V6, and R2 stages was 5%. Two glyphosate applications reduced pusley survival over that of a single application. Norsworthy (2005) and Murdock and Sherrick (1999) reported that Florida pusley control was dependent on plant size at the time of application, with control being inadequate when a single glyphosate application was delayed until the V6 to V7 soybean stage.

Light interception by soybean at 7 to 8 WAE was 77 to 80% in wide rows and 92 to 94% in narrow rows in both years (data not shown). Although soybean canopy effect on pusley survival was not significant across row widths, increased soybean competitiveness following canopy closure resulted in 84% average survival of nontreated plants by 11 WAE (Table 6.1). Norsworthy (2005) reported that reductions in the amount of available PAR following soybean canopy closure resulted in 84% mortality of Florida pusley (one of the pusley species in our study) beneath the canopy. The lack of a soybean canopy effect on survival of glyphosate-treated plants was because the experimental site was infested with a mixed population of Florida and Brazil pusley, and the latter is a perennial and can regrow vegetatively (Chandran and Singh 2003; Murphy et al. 1996), contributing to its ability to tolerate glyphosate. In other research, Brazil pusley was found to be a difficult-to-control weed with glyphosate (Chandran and Singh 2003; Sharma and Singh 2001).

Besides soybean competitiveness, intraspecific competition due to the high density of survivors in the nontreated control might have caused further mortality of pusley plants. At soybean harvest, survival of cohort 1 in the nontreated control was 76% compared to 23 to 28% following a single glyphosate application at the V3 or V6 stage (Table 6.1). Norsworthy (2005) reported that Florida pusley control with a single glyphosate application at 840 g ha^{-1} at the V3 and V6 stage provided no more than 95 and 66% control, respectively. In other research, Brazil pusley control was only 14% when glyphosate was applied at 770 g ha^{-1} (Sharma and Singh 2001). Glyphosate applications

at V3 and V6; or V3, V6, and R2 stages resulted in 1 to 3% pusley survival at soybean harvest, and thus, sequential glyphosate applications were superior to a single application, regardless of timing.

Pusley plants comprising cohort 2 emerged after the V3 glyphosate timing and were treated only at the V6 and R2 soybean stages. Survival of pusley plants in cohort 2 was not influenced by row width ($P = 0.3030$) and only the interaction of glyphosate timing and WAE on cohort 2 survival was significant ($P = 0.0002$). Pusley survival in cohort 2 at the V3 glyphosate timing was similar to the nontreated control because cohort 2 emerged after applying glyphosate at the V3 soybean stage. Survival 7 WAE in plots treated with glyphosate at the V6 timing ranged from 30 to 33% compared to 90 to 99% in the absence of glyphosate (Table 6.1).

Cohort 2 survival 9 WAE following glyphosate applied at the V6 and R2 soybean stages was 5% compared with 24 to 26% survival following a single glyphosate application. Intraspecific competition from cohort 1 resulted in density-dependent mortality of cohort 2, with survival averaging 66% by 13 WAE in the absence of glyphosate. At soybean harvest, survival of cohort 2 in the nontreated plots ranged from 47 to 55% compared to 14% following a single V6 application and an additional application at the R2 stage was needed to reduce survival to 1%.

Similar to cohort 1 and cohort 2, pusley survival in cohort 3 was affected by the interaction of glyphosate timing and WAE ($P = 0.0033$) and not influenced by row width ($P = 0.2616$). Survival of cohort 3 at one wk following the R2 glyphosate application was

26%. However, none of the treated plants survived through harvest compared with 49% average survival in the absence of glyphosate.

Pusley Vegetative Biomass

Pusley vegetative biomass production was influenced by the interaction of glyphosate timing and cohort ($P < 0.0001$) and was not affected by soybean row width. Total vegetative biomass production by pusley plants in the nontreated control was 309 g m^{-2} and was highest among all glyphosate treatments (Table 6.2). In the nontreated control, pusley plants from cohort 1 contributed 221 g m^{-2} of vegetative biomass compared with 69 and 19 g m^{-2} of vegetative biomass contributed by plants from cohort 2 and 3, respectively. This indicates that those plants that emerge with soybean are the most competitive and contribute more to total biomass similar to other weed species (Massinga et al. 2001; Norsworthy et al. 2007; Steckel and Sprague 2004).

Although pusley survival at the V3 timing in cohort 2 by soybean harvest was almost twice that of cohort 1 (Table 6.1), treated plants from cohort 1 produced vegetative biomass similar to plants from cohort 2 (emerged after the V3 application). Similarly, pusley plants from cohort 2 that survived a single application of glyphosate at the V6 stage produced biomass similar to those from cohort 3 (emerged after the V6 application). This provides further evidence that pusley species can tolerate a single glyphosate application, especially, Brazil pusley, due to its ability to regenerate vegetatively.

Furthermore, the data suggest that late emerging pusley cohorts, if not controlled, could contribute a significant amount of biomass.

Vegetative biomass production of pusley plants from cohort 1 that survived the single glyphosate application at the V6 stage was 83 g m^{-2}, and was 54% higher than the biomass produced by the plants that survived a single application at the V3 stage (Table 6.2). This was attributed to the larger size of pusley plants at the time of V6 application. Vegetative biomass production at harvest from cohort 2 plants that survived a single glyphosate application at the V6 stage was 13 g m^{-2}, which was 84% less than the biomass produced from cohort 1 plants. This was attributed to the larger size of pusley plants of cohort 1 by the V6 application.

In cohort 1, sequential glyphosate applications at V3 and V6 stages resulted in lower pusley biomass production compared to single applications at either V3 or V6. Similar biomass production from cohort 2 following a single application at the V6 stage or applications at V3 and V6 stages was expected because cohort 2 emerged after the V3 application. Pusley plants from cohort 3 produced 18 to 21 g m^{-2} of vegetative biomass in the absence of glyphosate. A third glyphosate application at the R2 soybean stage was needed to prevent pusley biomass production from cohort 3.

Pusley Seed production

Pusley seed production was influenced by the interaction of glyphosate timing and cohorts ($P < 0.0001$) and was similar across soybean row widths ($P = 0.0802$). Total seed

production by pusley plants was highest in the nontreated control (Table 6.2). Furthermore, pusley from cohort 1 produced 25,700 seeds m^{-2} in the absence of glyphosate, which was 68% of the total seed production compared with 11,800 seeds m^{-2} produced by cohort 2. Pusley plants from cohort 3 only contributed 360 seeds m^{-2}. This agrees with previous studies which showed weed cohorts that emerge at early crop stages and are not controlled produce more seeds than later emerging cohorts (Dieleman et al. 1996; Massinga et al. 2001; Norsworthy et al. 2007).

Besides similar biomass production by cohort 1 and 2 at the V3 timing, seed production by cohort 1 at the V3 timing was reduced and the survived pusley plants produced 3,900 seeds m^{-2} compared with 11,600 seeds m^{-2} produced by plants from cohort 2, which was expected because cohort 2 emerged after the V3 application. Pusley plants that survived or emerged after the single V3 glyphosate application produced a total of 15,870 seeds m^{-2}, which was more than twice the seed produced by plants following the single V6 glyphosate application. Delaying a glyphosate application to the V6 stage resulted in 4,500 seeds m^{-2} produced by pusley survivors from cohort 1 and was higher than the 2,290 seeds m^{-2} produced by those from cohort 2. Considering the low susceptibility of pusley to glyphosate, size of the plants at the time of application is crucial to reducing survival and seed production following glyphosate application (Murdock and Sherrick 1999; Reddy and Singh 1992; Sharma and Singh 2001).

At the V6 timing, although the biomass production from cohort 2 and 3 was similar, seed production by pusley plants from cohort 3 was numerically lower than that

from cohort 2. The data suggest that in addition to tolerance to a glyphosate application, early emergence would be an advantage for pusley species to increase seed production. Glyphosate applied at the V3 and V6 stages were needed to prevent seed production from cohort 1. Glyphosate applied at the V6 stage reduced pusley seed production of cohort 2 by 82% compared with the nontreated control. Seed production in cohort 3 that was not treated until the R2 stage was similar to the nontreated control. These results indicate that pusley cohorts emerging after the V3 soybean stage beneath the soybean canopy, if not controlled, can significantly contribute to the replenishment of the soil seed bank. Thus, an additional glyphosate application at the R2 stage was needed to prevent pusley seed production from cohort 2 and 3.

Palmer Amaranth Survival

The interaction of row width, glyphosate timing, and WAE was significant (P = 0.0068) for Palmer amaranth survival of cohort 1. Survival 2 wk following glyphosate applied at the V3 soybean stage was 6 or 7% regardless of row widths (Table 6.3). Even if interaction was significant, the pattern of survival was similar across row widths. A single glyphosate application at the V6 stage resulted in a 91 to 97% reduction in Palmer amaranth survival by 7 WAE. Survival 2 wk following a single glyphosate application at the V6 stage was similar to sequential applications in narrow rows. In wide rows, glyphosate applied at the V3 and V6 stages resulted in 2% survival, which was less than the 9% survival following a V6 only application. Further mortality occurred in narrow

rows following soybean canopy closure, with no survivors from cohort 1 in any narrow-row, glyphosate-treated plot by 9 WAE. Thus, a single glyphosate application at the V3 or V6 stage was sufficient to prevent survival of Palmer amaranth plants that emerged from soybean planting through V3 stage in narrow rows.

Effects of single or sequential glyphosate applications on cohort 1 survival were also similar in wide rows by 9 WAE; however, Palmer amaranth survival averaged 1% through harvest following a single V3 or V6 application in wide rows. In a study conducted by Young et al. (2001) examining the effect of soybean row spacing and glyphosate timing on control of common waterhemp, a closely related *Amaranthus* species, it was reported that control 5 wk after a single mid-season glyphosate application at a similar rate was 90 and 99% in 76- and 19-cm soybean row widths, respectively. Norsworthy (2004) also reported that Palmer amaranth control 9 WAE in wide-row soybean was 100% following single or sequential glyphosate applications; however, the present study indicates that a followup glyphosate application at the V6 soybean stage might be important in wide rows to prevent survival of those plants that escaped a single application at the V3 stage. Palmer amaranth plants emerging at early stages of the crop are highly competitive and 99% control may result in densities as low as 0.5 to 1 plant m^{-1} of row which has been shown to cause yield loss in soybean (Klingaman and Oliver 1994; Massinga et al. 2001).

Differences in canopy closure between row widths and competitiveness of soybean and pusley with Palmer amaranth was evident based on mortality of Palmer

amaranth in the nontreated control. Cohort 1 survival at harvest in the nontreated control was 67% in wide rows compared with 44% survival in narrow rows. Narrowing the soybean row spacing appears to be one strategy producers can utilize to minimize Palmer amaranth survival, which is important considering its reproductive potential which is up to 600,000 seeds per female plant (Keeley et al. 1987; Massinga et al. 2001; Sellers et al. 2003). The benefit of earlier canopy closure in narrow compared with wide rows for improving common waterhemp control has been previously documented (Young et al. 2001).

In cohort 2, Palmer amaranth emergence was inconsistent and minimal over the experimental area in both years because of interspecific competition for space and light from the mixed population of Florida and Brazil pusley which had high emergence and survival rate. Furthermore, it is likely, especially in the nontreated controls, that Palmer amaranth from cohort 1 contributed greatly to the mortality of the later emerging cohort 2. Averaged over years and row widths, cohort 2 survival in the absence of glyphosate ranged from 15 to 20% (data not shown). Effect of delayed emergence on reduced competitiveness and survival of *Amaranthus* species in soybean and corn has been documented. Mortality of common waterhemp that emerged between the V3 to V6 stage of soybean or corn was up to 97%, irrespective of row width (Hartzler et al. 2004; Nordby and Hartzler 2004; Steckel and Sprague 2004). None of the Palmer amaranth from cohort 2 survived a single glyphosate application at the V6 stage (data not shown) likely because

of glyphosate's effectiveness as well as interference with soybean and the earlier emerging Palmer amaranth and pusley cohort.

Palmer Amaranth Vegetative Biomass

The interaction of row width and glyphosate timing was significant for Palmer amaranth vegetative biomass production from cohort 1 ($P = 0.038$). The nontreated control in 19- and 97-cm rows produced greater vegetative biomass than following any of the glyphosate treatments. Averaged over years, biomass production from cohort 1 in the nontreated control was 190 g m^{-2} in wide rows compared to 120 g m^{-2} in narrow rows, a 37% decrease (Table 6.4). Lower biomass production in narrow rows was due to greater reduction in the amount of available PAR and greater soybean competitiveness in narrow compared with wide rows (Dalley et al. 2004; Norsworthy et al. 2007; Steckel and Sprague 2004). A single glyphosate application at the V3 or V6 stage was sufficient to prevent Palmer amaranth vegetative biomass production from cohort 1 in narrow rows but not in wide rows where 3.3 and 1.3 g m^{-2} of vegetative biomass was produced following a single glyphosate application at the V3 and V6 stage, respectively. This biomass production would be greater than that reported here if soybean had not been harvested in a timely manner. Delays in soybean harvest following maturity due to weather or other time constraints would result in greater biomass production as well as increasing the likelihood of seed production by these surviving plants.

Averaged over years, vegetative biomass production from cohort 2 in the nontreated plots ranged from 8 to 12 g m^{-2}, which was almost 93 to 94% less than the vegetative biomass produced by nontreated plants from cohort 1. Thus, the effect of interspecific and intraspecific interference on reduced Palmer amaranth growth was evident, which is consistent with findings by others that biomass production of *Amaranthus* species emerging after the V3 stage of soybean or corn is reduced up to 97% (Hartzler et al. 2004; Knezevic and Horak 1998; Massinga et al. 2001; Nordby and Hartzler 2004).

Palmer Amaranth Seed Production

Palmer amaranth seed production from cohort 1 was influenced by the interaction of row width and glyphosate timing ($P = 0.023$). Seed production from cohort 1 was greater in the nontreated control compared to any of the glyphosate application treatments. Averaged over years, nontreated Palmer amaranth from cohort 1 produced 211,400 seeds m^{-2} in wide rows compared to 139,400 seeds m^{-2} in narrow rows (Table 6.4). The reduced seed production in narrow rows was due to early canopy closure and greater competitiveness of narrow- compared with wide-row soybean (Dalley et al. 2004; Norsworthy et al. 2007; Steckel and Sprague 2004). Palmer amaranth from cohort 1 in wide rows that survived the V6 glyphosate application failed to produce seed. However, Palmer amaranth that survived glyphosate applied at the V3 stage in wide rows produced 600 seeds m^{-2}, which would contribute to the replenishment of Palmer amaranth in the

soil seed bank. Hence, sequential glyphosate applications in wide-row soybean are needed to prevent Palmer amaranth seed production when the first application is made at the V3 soybean stage.

Averaged over years and row widths, Palmer amaranth from cohort 2 in the nontreated plots produced 5,600 seeds m^{-2}, a 97% average decrease from cohort 1 (data not shown). Massinga et al. (2001) reported that Palmer amaranth emerging with corn produced 140,000 seeds m^{-2} compared with 18,000 seeds m^{-2} when emerging from the V4 to V7 corn stages. A single glyphosate application at the V6 soybean stage prevented Palmer amaranth survival, biomass, and seed production from cohort 2 (data not shown).

Soybean Yields

The main effects of glyphosate timing ($P < 0.0001$) and soybean row width ($P = 0.0076$) were significant for soybean yield and no interactions were significant. Averaged over years and row widths, soybean yields following sequential glyphosate applications were higher and ranged from 2490 to 2640 kg ha^{-1} compared to 1850 to 2020 kg ha^{-1} following single glyphosate applications at the V3 or V6 stage (Figure 6.1). Thus, a single glyphosate application was not sufficient to prevent soybean yield losses from a mixed population of pusley and Palmer amaranth. Soybean yield reduction averaged 73% across row widths in the nontreated control compared with the treatment receiving three glyphosate applications, evidence of the high degree of weed interference. Averaged over

years and glyphosate timings, soybean yield was 2260 kg ha^{-1} in narrow rows, which was 38% higher than the yield of 1630 kg ha^{-1} in wide rows (data not shown).

Based on the results, it is concluded that two early-season glyphosate applications are needed to minimize pusley and Palmer amaranth survival and maximize soybean yields. Pusley species have an extended period of emergence and pusley cohorts that emerge from soybean planting through the V6 stage are highly competitive and have high biomass and seed production potential if not controlled. Furthermore, pusley plants that emerged after the V6 stage survived through soybean harvest irrespective of row width and produced up to 430 seeds m^{-2}, further evidence of the difficulty in managing pusley using a glyphosate-only approach in glyphosate-resistant soybean.

Palmer amaranth survival was mainly attributed to the plants that emerged in cohort 1 from soybean planting (last wk of June) through the V3 stage (mid- to late July) in both years. The emergence of cohort 2 that occurred from the V3 to V6 stage (early August) was minimal, and no emergence occurred after the V6 soybean stage. Palmer amaranth biomass and seed production from cohort 1 in the absence of glyphosate was higher in 19-cm compared with 97-cm rows due to greater light interception and greater soybean competitiveness in narrow rows. Like other C_4 species, *Amaranthus* species have a competitive advantage under conditions of high light intensities and have less tolerance to shade compared with C_3 species that are better adapted to shading by a crop canopy (Stoller and Myers 1989). Future research is needed on the effect of prolonged shading on survival, growth, and fecundity of Palmer amaranth and pusley species.

A single glyphosate application was sufficient to prevent Palmer amaranth survival, biomass, and seed production in narrow rows, but plants that survived an early-season glyphosate application in wide rows produced seeds. This warrants the need for multiple glyphosate applications or the use of an effective residual herbicide. This research provides evidence that two applications of glyphosate are needed to protect against soybean yield loss from a mixed population of Palmer amaranth and pusley species in glyphosate-resistant soybean.

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Table 6.1. Percentage survival of three pusley cohorts averaged over 19- and 97-cm-row widths and years (2004 and 2005) as influenced by glyphosate application timing during the soybean growing season at Blackville, SC.

		Pusley survival[a]						
Cohort	Timing[b]	5 WAE[c]	7 WAE	9 WAE	11 WAE	13 WAE	15 WAE	17 WAE
		%						
Cohort 1	V3	41 b A[d]	31 c B	27 b BC	26 b C	24 b C	23 b C	23 b C
	V6	100 a A	46 b B	36 b C	32 b CD	31 b D	28 b D	28 b D
	V3/V6	30 c A	7 d B	5 c B	4 c B	3 c B	3 c B	3 c B
	V3/V6/R2	37 bc A	14 d B	5 c C	2 c C	1 c C	1 c C	1 c C
	None	100 a A	97 a A	90 a B	84 a C	77 a D	77 a D	76 a D
Cohort 2	V3		90 a A	80 a B	72 a B	56 a C	51 a C	47 a C
	V6		30 b A	24 b ABC	20 b BC	17 b BCD	14 b D	14 b D
	V3/V6		33 b A	26 b B	20 b BC	17 b BC	14 b C	14 b C
	V3/V6/R2		31 b A	5 c B	1 c B	1 c B	1 c B	1 c B
	None		99 a A	90 a A	77 a B	66 a C	57 a D	55 a D
Cohort 3	V3			86 a A	80 a AB	61 a BC	58 a C	57 a C
	V6			81 a A	69 a A	61 a AB	47 a B	47 a B
	V3/V6			81 a A	62 a AB	47 a B	45 a B	45 a B
	V3/V6/R2			26 b A	7 b AB	1 b B	0 b B	0 b B
	None			86 a A	67 a AB	56 a B	50 a B	49 a B

[a] Cohort 1 comprised pusley emerging after soybean planting through the V3 soybean stage (3 WAE). Cohort 2 comprised pusley emerging after the V3 stage through the V6 soybean stage (5 WAE). Cohort 3 comprised pusley

Table 6.1 (Continued)

emerging after the V6 stage through the R2 soybean stage (8 WAE). Survival of the three cohorts were analyzed separately.

[b] Glyphosate at 840 g ae ha^{-1} was applied at V3 [(3 wk after soybean emergence (WAE)]; V6 (5 WAE); V3 and V6; and V3, V6, and R2 (8 WAE) soybean stages. No glyphosate was applied to the "None" timing.

[c] WAE indicates wk after soybean emergence over which survival of the cohort was monitored.

[d] For each cohort, means within a column followed by the same lowercase letters and means within a row followed by the same uppercase letters are not significantly different based on Fisher's protected LSD test at =0.05.

Table 6.2. Vegetative biomass and seed production of three pusley cohorts averaged over 19- and 97-cm soybean row widths and years (2004 and 2005) as influenced by glyphosate application timing at Blackville, SC.

	Pusley biomass[a]				Pusley seed production			
Timing[b]	Cohort 1[c]	Cohort 2	Cohort 3	Total	Cohort 1	Cohort 2	Cohort 3	Total
	g m^{-2}				No. m^{-2}			
V3	54 c A	61 a A	18 a B	133 b	3900 b A	11600 a B	370 a C	15870 b
V6	83 b A	13 b B	21 a B	117 b	4500 b A	2290 b B	390 a B	7180 c
V3/V6	4 d A	12 b A	20 a A	36 c	0 c A	1980 b B	430 a AB	2410 d
V3/V6/R2	1 d A	0.5 b A	0 a A	1.5 c	0 c A	0 c A	0 a A	0 d
None	221 a A	69 a B	19 a B	309 a	25700 a A	11800 a B	360 a C	37860 a

[a] Cohort 1 comprised pusley emerging after soybean planting through the V3 soybean stage [3 wk after soybean emergence (WAE)]. Cohort 2 comprised pusley emerging after the V3 stage through the V6 soybean stage (5 WAE). Cohort 3 comprised pusley emerging after the V6 stage through the R2 soybean stage (8 WAE).

[b] Glyphosate at 840 g ae ha^{-1} was applied at V3 [(3 wk after soybean emergence (WAE)]; V6 (5 WAE); V3 and V6; and V3, V6, and R2 (8 WAE) soybean stages. No glyphosate was applied to the "None" timing.

[c] Means within a column followed by the same lowercase letters and means within a row followed by the same uppercase letters are not significantly different based on Fisher's protected LSD test at =0.05.

Table 6.3. Percentage survival of Palmer amaranth from cohort 1 averaged over years (2004 and 2005) as influenced by glyphosate application timing and row width during the soybean growing season at Blackville, SC.

		Palmer amaranth survival[a]				
		Cohort 1				
Row width	Timing[b]	5 WAE[c]	7 WAE	9 WAE	11 WAE	13 WAE
		%				
19 cm	V3	7 b A[d]	3 bc A	0 b B	0 b B	0 b B
	V6	100 a A	3 bc B	0 b B	0 b B	0 b B
	V3/V6	8 b A	1 c B	0 b B	0 b B	0 b B
	V3/V6/R2	8 b A	2 c AB	0 b B	0 b B	0 b B
	None	100 a A	96 a A	72 a B	47 a C	44 a C
97 cm	V3	6 b A	3 bc AB	1 b B	1 b B	1 b B
	V6	100 a A	9 b B	3 b C	2 b C	1 b C
	V3/V6	12 b A	2 c B	0 b B	0 b B	0 b B
	V3/V6/R2	7 b A	2 c AB	0 b B	0 b B	0 b B
	None	100 a A	98 a A	85 a B	70 a C	67 a C

[a] Cohort 1 comprised Palmer amaranth emerging after soybean planting through the V3 soybean stage (3 WAE).

[b] Glyphosate at 840 g ae ha^{-1} was applied at V3 [(3 wk after soybean emergence (WAE)]; V6 (5 WAE); V3 and V6; and V3, V6, and R2 (8 WAE) soybean stages. No glyphosate was applied to the "None" timing.

[c] WAE indicates wk after soybean emergence over which survival of the cohort was monitored. [d] Means within a column followed by the same lowercase letters and means within a row followed by the same uppercase letters are not significantly different based on Fisher's protected LSD test at =0.05.

Table 6.4. Palmer amaranth vegetative biomass and seed production from cohort 1 averaged over years (2004 and 2005) as influenced by glyphosate application timing at Blackville, SC. Cohort 1 comprised plants that emerged from soybean planting through the V3 soybean stage.

Row width	Timing[a]	Biomass[b]	Seed production
		g m^{-2}	No. m^{-2}
19-cm	V3	0 c	0 c
	V6	0 c	0 c
	V3/V6	0 c	0 c
	V3/V6/R2	0 c	0 c
	None	120 b	139400 b
97-cm	V3	3.3 c	600 c
	V6	1.3 c	0 c
	V3/V6	0 c	0 c
	V3/V6/R2	0 c	0 c
	None	190 a	211400 a

[a] Glyphosate at 840 g ae ha^{-1} was applied at V3 [(3 wk after soybean emergence (WAE)]; V6 (5 WAE); V3 and V6; and V3, V6, and R2 (8 WAE) soybean stages. No glyphosate was applied to the "None" timing.

[b] Means within a column followed by the same letter are similar based on Fisher's protected LSD test at =0.05.

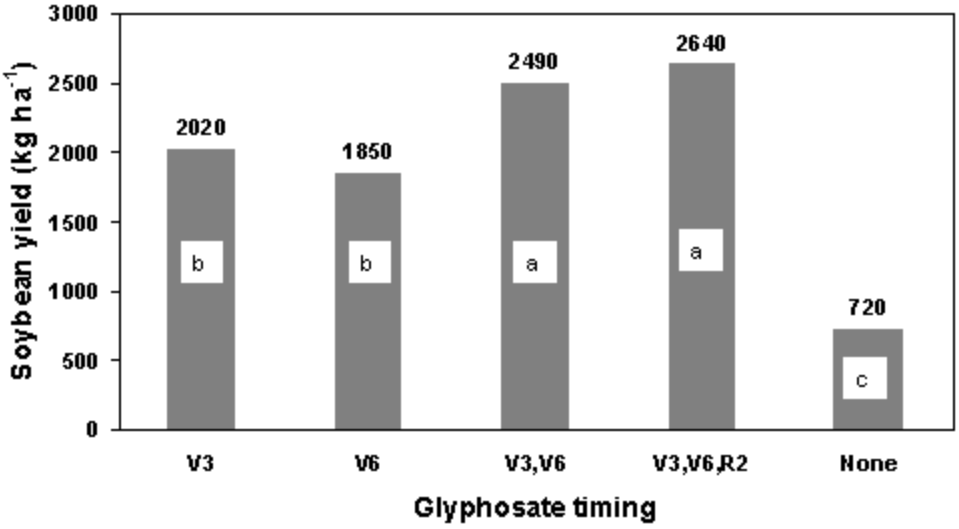

Figure 6.1. Soybean seed yield averaged over 19- and 97-cm-width rows and years (2004and 2005) as influenced by glyphosate at 840 g ae ha^{-1} applied at the V3; V6; V3 and V6; and V3, V6, and R2 soybean stages and a nontreated control. Means having the same letter are similar based on Fisher's protected LSD at = 0.05.

CONCLUSIONS

Studies were performed on different aspects of Palmer amaranth biology and ecology with the aim of improving knowledge on the population and seedbank dynamics of Palmer amaranth. It is one of the most problematic weeds in southern United States, especially with the evolution of Palmer amaranth biotypes resistant to multiple herbicide chemistries including glyphosate.

Temperature and light play important roles in mediating seasonal periodicity in germination and emergence of Palmer amaranth from a soil seedbank. Following dormancy reduction of seeds during winter, germination was favored by temperatures between 25 and 35 C and thermal amplitudes of 15 C. These temperature conditions occur in South Carolina during late spring to summer, which result in two to three major emergence periods from early May through mid-July. Soil moisture following a rainfall event favors the occurrence of these emergence flushes. In addition, magnitude and duration of emergence are influenced by the rainfall distribution during a season. Emergence is of greater magnitude with shorter duration during a wet season, whereas it is of smaller magnitude and longer duration during a dry season. Peak emergence periods of Palmer amaranth observed in this research coincided with the recommended planting dates for cotton and soybean in South Carolina, explaining the severity of infestation of Palmer amaranth in these crops.

In the fall (August and November), Palmer amaranth seeds showed a phytochrome mediated response to red (R) and far-red (FR) light exposures, with a germination

enhancement by R light and inhibition by FR light. Germination was also lower in dark compared to natural light conditions. A decrease in photosynthetic active radiation (PAR) and R:FR ratio beneath the soybean canopy following initiation of canopy closure by July resulted in significant reductions in Palmer amaranth emergence in the field even when temperature conditions were favorable. Palmer amaranth emergence in South Carolina ceased by late fall (October and November), when the temperature ($\geq$25 C mean) requirement for germination was unlikely to occur.

Reduction in germination of Palmer amaranth seeds following burial in spring suggested that depth-mediated dormancy is a mechanism for survival and persistence of seeds in the soil seedbank. A natural light or R light requirement for germination of buried seeds further implied that buried seeds would germinate following soil disturbance by tillage or planting operations in the following spring. No difference in emergence was observed between no-tillage and shallow spring tillage conditions in the field.

Differences in maternal light (PAR) environment during the development of seeds had a significant impact on timing and extent of seedling emergence of Palmer amaranth within a population. Seeds of plants maturing under moderate shading (up to 47% reduction in PAR) showed germination response similar to the seeds maturing in direct sunlight. Increased dormancy of seeds maturing under 87% shade conditions would aid in survival for seeds developing and maturing beneath a crop canopy. .

Palmer amaranth seeds produced by a single plant can also exhibit differential dormancy, dormancy was greater for seeds maturing in the bottom third location of the

mother plant. This implied that Palmer amaranth seeds showed leaf-canopy inhibited germination. Seeds maturing in the bottom third of the plant or under 87% shade had higher ABA levels, implying that ABA production is triggered in seeds maturing under reduced light levels. Differences in GA and ABA; however, did not explain the variability in dormancy of Palmer amaranth seeds within a population or a single plant. Seed weight was not affected by maternal shading or seed position on the plant. These results indicated that germination ability of Palmer amaranth was reduced under low light conditions which would allow the seeds to survive and germinate later when growth conditions were more favorable.

High light-saturated photosynthetic rates and high growth rates help explain the competitiveness of Palmer amaranth in high-light intensity environments. Besides the adaptive mechanism of dormancy induction under shaded environment, Palmer amaranth shows photosynthetic and morphological acclimation to shading (up to 87% reduction in PAR) by decreasing light-saturated photosynthetic rates, dark respiration, leaf and main stem branch appearance rates, and by increasing specific leaf area or leaf chlorophyll content or both. By being able to adapt to changing light conditions, Palmer amaranth is able to survive and reproduce in a changing environment. This may help explain the importance of Palmer amaranth as a troublesome weed in crops.

In a glyphosate-resistant cropping system, an early season application of glyphosate preferably at the V3 stage of soybean (18-cm narrow rows) is needed to prevent survival and seed production of Palmer amaranth from cohorts emerging early in

the growing season. Plants escaping the single V3 application are capable of producing seeds in wide rows (97-cm wide) and warrants the need for a sequential herbicide application. Based on this research, narrow row spacing is a strategy which can improve Palmer amaranth control, especially from cohorts emerging after the V3 stage; although the emergence of Palmer amaranth from a natural seedbank in South Carolina is minimal (<10% of the total emergence during a season) following V6 stage (July) of soybean.

Two to three sequential applications of glyphosate at the V3, V6, and R2 stages of glyphosate-resistant soybean were needed to control pusley species (Florida and Brazil pusley), which were present along with Palmer amaranth in a naturally infested test site used in one of the studies of Palmer amaranth. Pusley species exhibited an extended period of emergence beginning from soybean planting through the R2 stage. Pusley emerging between the V6 and R2 stages were the least competitive, but contributed 379 seeds m^{-2} in the absence of glyphosate, which can be significant for replenishment of the soil seedbank. Further attention is needed especially when multiple glyphosate applications are required to prevent biomass and seed production of these species.

Further research is needed to understand the interaction of additional environmental factors in controlling seed germination and emergence of Palmer amaranth. Research is needed to investigate the interaction of temperature, light quality (R:FR ratio), photoperiod, moisture, and nutrient availability on maternal regulation of seed dormancy. The role of endogenous hormones including ABA and GA in mediating seasonal changes in germination response of seeds also needs to be further investigated.

These biology and ecology studies presented for Palmer amaranth need to be integrated into developing simulation models for predicting emergence and crop-weed competition under various production scenarios especially in relation to crops such as soybean and cotton, where Palmer amaranth has been shown to be a significant problem.